DRUG INFORMATION ASSOCIATION

FDA/DIA SCIENTIFIC WORKSHOP ON FOLLOW-ON PROTEIN PHARMACEUTICALS

PLENARY SESSION

Monday, February 14, 2005

8:00 a.m.

Marriott Crystal Gateway
1700 Jefferson Davis Highway
Arlington, Virginia

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C O N T E N T S

P R O C E E D I N G S

MR. WEBBER: It is time to get this meeting underway. If we could everyone to, please, enter the room, find your seats, get settled, we'll get started on two and a half days of what I am sure are going to be very interesting talks and discussions.

Good morning. I welcome everyone to the FDA/DIA Scientific Workshop on Follow-On Protein Pharmaceuticals.

I am Keith Webber. I am currently the acting director of the Office of Biotechnology Products in CDER, and one of the co-chairs for the organizing of this committee.

Before we get started, I would especially like to thank the Planning Committee. Certainly, this was a major endeavor to put this meeting together, I think. I would like to thank my other co-chairs, Chi Wan Chen and Chris Joneckis, who did a great deal to help put this together, as well as, and certainly not any less, the members of the committee listed here.

I will go through the list, just to be sure that everybody gets recognition.

Janice Brown, Barry Cherney, Kathleen

Clouse, Clair Fraser, Dena Hixon, Frank Holcombe, Steve Kozlowski, Stephen Moore, Amy Rosenberg, and Marilyn Welschenbach.

Those are all folks from the agency who worked on the committee.

But in addition, we had industry representatives who really did a lot, as well, and equally, if not more, to put together the list of speakers and working on the breakout sessions.

Terry Gerrard, Gordon Johnston, Tony Lubiniecki, Gene Murano, Sara Radcliffe, and Marie Vodicka.

In addition, of course, the DIA, I would like to thank Jessica Kusma and Joe McNair, who really did a huge amount to put the meeting together, taking care of the logistics of managing such a large crowd and such a complicated meeting, with as many breakout sessions as we have.

I'm not going to go through this list, but

certainly do want to thank those who volunteered to help by speaking or participating in the plenary sessions and the breakout sessions. It is a huge list of people that you will come to know and come to hear and talk with over the next two and a half days.

This is the second in a series or pair of scientific workshops that the agency is holding to gather input and information in preparation for drafting guidance in the area of follow-on protein pharmaceuticals.

The first was in September, a two-day meeting, the 14th and 15th, and this meeting was really open to all-comers, kind of an open mic forum, where anyone who wished, any stakeholder could come and speak, give us their input in this area of public policy.

We did hear from 23 interested parties at that meeting. We got comments and recommendations in areas of terminology for these types of products, the evaluation of product complexities, analytical methods that can be used to assess these

products, clinical trial design, as well as design of immunogenicity studies and issues related to immunogenicity for protein products.

For those who may not know, this is another opportunity to tell you, the transcripts for that meeting are available on the FDA docket, which is listed here. It's Number 2004N0355.

Now, this meeting is going to be a bit different than the last meeting. This is more for discussion, more for really a working meeting, I would call it, and I think that everybody probably will get a lot of work done here in this area.

We are going to have morning plenary sessions and then in the afternoons of today and tomorrow there will be focused breakout sessions for discussion.

The summary presentations of the breakout sessions will be presented on the following day. So tomorrow we will hear summaries in tomorrow's plenary sessions for today's breakout sessions. On Wednesday, we will hear summaries of tomorrow's breakout sessions. So everybody will be able to

get sort of a recap of all the discussions.

The plenary sessions include invited speakers, which the committee, primarily, industry folks selected to come and speak on these topics. They are to serve as a background for breakout session discussions in the afternoon.

So I would say just try to reserve questions for this morning's speakers for further discussions at the breakout sessions.

We have asked the speakers to generally focus on protein products of varying levels of complexity, so that we are not all focused on those of high complexity or those of low complexity, but to give a range and cover as much breadth as one can in those talks.

For the breakout sessions, each of the topics will be repeated twice, in two sessions. During lunch, they are going to break this room into four rooms and that will start, I believe, at A, B, C and D breakout sessions, and the topics for those are in the program.

For each breakout session, there is going

to be an FDA lead and two industry moderators, and then the moderators, including the FDA lead, are going to solicit discussion from the audience via focused questions, so that we can really try to get out of those sessions the most information we can on the most pertinent questions.

Just sort of some ground rules for the breakout sessions. If you are going to speak, and we certainly encourage you to speak, speak in the microphones, but try to keep comments as concise as you can, because we have a lot of people here and each breakout session is only going to be about an hour and a half.

So if you can keep your comments concise, to about two minutes, at most, and keep them relevant to the topic under discussion. If someone else wants to, if you have a comment that addresses something slightly different and there is discussion on a particular topic, maybe you could wait just until that discussion is over and then move on to your topic or your question or comment.

For the transcribers, if you can have a

business card available when you go to the microphone, we will have someone to collect those and then the transcriber can have those to help with writing the names and the affiliations for the speakers.

Today's agenda, we have a keynote speaker, Charles Cooney, and then we will have a background presentation by Stephen Kozlowski from the FDA.

There are going to be two plenary sessions, one on physicochemical and biological characterization, and then a session on pharmacokinetics and pharmacodynamics studies.

At that point, we will break for lunch and then come back for the concurrent breakout sessions in the afternoon on these topics.

Tuesday's agenda, as I said, we will have the breakout session summaries in the morning. We will only do three of those breakout sessions, just in the interest of maintaining time. So it will be the chemical and biological characterization sessions and the pre-clinical pharm talk session.

We will have two plenary sessions, one on

immunogenicity studies, one on clinical safety and efficacy, and then, again, in the afternoon, we will have concurrent breakout sessions on those topics.

For Wednesday, we will do the breakout session summaries in the morning and then we will have two summaries by industry, one from BIO/PHRMA, combined, and one from GPHA, and then I will give a summation, shortly, of the entire meeting and then we will have some closing remarks from Dr. Ajaz Hussain, and then next steps with regard to the guidance development process.

If you have additional information or data from your presentations or discussions during the breakout sessions that you would like to include in the record, you can submit those to the same docket that I mentioned before; that is, Docket Number 2004N-0355. The zero is important, too, don't forget that.

That docket was reopened for the purposes of collecting additional information. Also, transcripts of this meeting will be available as

soon as possible afterwards.

Now, without further ado, since we got started a little bit late, I would like to start right off with introducing our keynote speaker, who is Dr. Charles Cooney, from Massachusetts Institute of Technology. He is a Professor of Chemical and Biochemical Engineering there, as well as a co-Director of the Program on the Pharmaceutical Industry.

As well, he has volunteered to be our Chair of the Advisory Committee for Pharmaceutical Sciences at the FDA.

So I would like you to welcome Dr. Cooney.

[Applause.]

DR. COONEY: Thank you very much, Keith. I am pleased to be here this morning. As I look around the room, I see many faces of individuals that I have had a chance to work with over the last 30-plus years in various aspects of developing biochemical processes for making a wide variety of products, and it is a pleasure to be back here with many of you.

It is also a privilege to have the opportunity to provide some opening comments this morning to try and help in making a contribution to

the framework for the discussion that will take place in the next two and a half days.

The real work of the meeting, the real opportunity is in those next two and a half days, when a lot of relevant topics are going to come up.

Now, many of you who know me know that I have a passion for things like risk and uncertainty, and there are some themes that I think are relevant to that passion and that are relevant to the discussion today.

When one looks at uncertainty, when one looks at risk, it is important to realize that this is risk that is not necessarily--it can't be avoided, if you want to get to the end point, and it's a risk that one actually wants to embrace.

In fact, as you look at that risk and as we look at the risks in the next couple of days, we want to keep in mind the theme that these risks can be managed. We need to know what they are, we need

to know where they are, we need to know how to measure them, but these are things that we can manage and we can work through.

As we look at this workshop, it is really about the scientific foundation for developing, evaluating, producing, delivering follow-on therapeutics, and, in particular, proteins, which is the focus of these couple of days.

The goal that we have, and we will talk a bit about goals, is to be able to assess the uncertainty and risks and to embrace that and manage it, to learn how to manage it and to do so in an appropriate way.

But, of course, the challenge we have in facing this goal is to try and reach a consensus for the path to follow to assure safe and efficacious follow-on products in an environment in which there are many multiple agendas.

This, indeed, is a challenge and it is one that I believe that this group is going to be up to. As we look at the goal, it is important to keep in mind a couple of concepts.

Now, the goal this morning was to reach the Crystal City Marriott. You were able to do so because you knew where you wanted to go and you had

some metric that allowed you to measure where you were at any given time.

It might have been time, it might have been place.

Now, certainly, Vasco Da Gama went around the Cape of Good Hope in 1497, he sort of knew where he wanted to go, but his metrics were much more primitive. So the path was a bit more challenging. Yet, those goals are the ones that we really face today and the one thing that I suggest that we keep in mind is that if we don't know where we are going and if we don't have a means of measuring where we are, then we will never know when we arrive, and we will come back to this a number of times.

A couple of other key concepts that I have found to be very useful in my experiences in biotech processes over the last couple of decades, simply summarized, one of the most important is

really the analytical technology and the tools, because it is the availability of these technologies that define where we are, and we need to know where we are.

It is knowledge that we need to bring together. This is the information. This is what we have learned, and, of course, that is driven by the analytical technologies. It is constrained by the analytical technologies that we have available to us.

What is it that brings us here now? Well, a lot of it is around uncertainty. This is a measure of how well we know something or perhaps a measure of how well we don't know it, but nonetheless, we know that an uncertainty is there and it is the uncertainty that drives our concern about hazards and that drives our concern about risk.

So if we can come to grips with that uncertainty through knowledge generated by analytical tools, perhaps we can make some progress going forward, and, of course, the desired outcome

is where we want to go.

If we don't know where we want to go and we don't have a means of measuring where we are, then we aren't going to know when we arrive.

So when you think about, well, what are follow-on biologics, and, of course, it depends on your perspective, and many of you do have different perspectives and different agendas.

Well, let me start with a perspective of what I think the state is today, where are we today, and we, in fact, have multiple processes and multiple manufacturers for a variety of biological products. That is the reality of today. And a number of manufacturers make the same product in the multiple locations or they make the same therapeutic protein at multiple locations.

And when we look at the processes that are being developed by you and your colleagues for follow-on products, we acknowledge that they are likely to be different than the processes that are in use today, and, of course, there is this connection between process and product that we need

to understand.

But why are those processes going to be different? Why can't they be the same? Well, there are a number of reasons. First of all, issues of intellectual property and freedom to operate prevent many follow-on manufacturers from practicing the same processes used by others.

Different analytical methodologies. This can be a choice, that we choose to use a different technique or methodology, or it may be that the methodologies that we use today and tomorrow weren't available five, ten, twenty years ago.

I recall the very dramatic example of when the gold standard for purity on a gel was a Coomassie blue stain, and then, of course, when silver stain evolved, we realized how dirty those gels were.

So there are advancements that take us forward in improving our knowledge.

Of course, incorporation of prior knowledge into new processes, whether it be innovation into new process technology, there are

new methods increasingly available, or the use of information, largely from the public domain, from not only existing products, but related products, is very important and defines the experimental space, defines the operating space that we are going to work in.

But as you go down that path, it, indeed, can be a lonely path, but we need to remember that you need to know where you are going and you need to have a measure of where you are.

Well, what is going to drive us down this path? What are the drivers for the interest in follow-on biologics? There are several.

Certainly, the reduced cost of follow-on biologics or certainly the perception that there will be reduced costs for follow-on biologics, and this is very much a political agenda. There is as business opportunity. When you look at the U.S. revenue alone for these products, they are $40 billion, and with a very high gross margin. So there is a lot of incentive to be in that business. And expanding patient care; expanding patient care

through new products, but certainly through availability of additional products and to new markets is yet another driver.

So there are a lot of incentives in order to pursue what is a very important question.

Well, the problem. What is the problem that we face that has pulled us together here? Well, largely, it revolves around the inadequate definition of molecular complexity, and this molecular complexity, which is a continuum, it's not a singular state, is associated with the structure and function of these protein pharmaceuticals.

Well, why is there molecular complexity? Well, one could take the philosophical role and try to approach that. I will avoid that one.

But from a practical point of view, there are barriers that really are barriers to understanding what that complexity is, and that is what we need to come to grips with; the analytical methods, the definition of the process space in which we manufacture these products, and, of

course, understanding the relationships between how we make a molecule, its structure, and, ultimately, its function.

Reminding us that in the European authorities, in recognition of molecular complexity, I came to the conclusion, in a concept paper, that the generic approach is scientifically not appropriate and a bio approach, based on some aspects of comparability, needs to be based on science and acceptable methods, and that's what we're doing today.

Well, let's come back to this theme of uncertainty. Uncertainty is the foundation of risk, whether it be real or perceived. Therefore, we need methods that will reduce that uncertainty. We need methods and approaches that will allow us to manage the risk, because there is always going to be risk there.

The only way to avoid risk is not to go into the mountain in the first place. But, in fact, we have all chosen to go on to that mountain, so we need to manage it and embrace it.

So we need to know where we want to go and be able to measure where we are.

Now, molecular complexity takes on many

different shapes and sizes. The common feature of all the molecules represented here are that they are all made by a biological process, whether it be penicillin, in the upper right-hand corner, an antibody, or a recombinant protein, such as alpha-1 antitrypsin, and it is understanding that complexity that we all aspire to do.

When we look at the relationship of or look at the scale of complexity and our ability to characterize it across that spectrum, we see several things.

On the vertical axis, if we look at the ability to characterize, and then, on the horizontal axis, relative to the purity of the molecule relative to the complexity, the more pure the molecule, the less the number of species and the easier it is to characterize the preparation.

But this particular line recognizes the fact that many of the products that we make by

these technologies are, in fact, not necessarily pure, but that is clearly an important issue.

When we look at molecular complexity, which may have to do with size, shape, whether it's a homodimer or a heterodimer, post-translational modification and the like, as that complexity goes up, it, not surprisingly, becomes more difficult to characterize.

So the space where we operate today is largely with molecules with relatively low to modest complexity, because we can embrace those materials, and relatively high purity.

But when we look forward as to where we are going to go over the coming years, we want to be able to span this entire space, and that is the challenge.

As we look at probing molecular complexity, we have a variety of options and strategies. What are the relevant structural features?

Of course, these are some of the questions that you will need to embrace in the discussion

later today.

What are the relevant structural features? What are the purity requirements? What are the attributes of those materials that are important in terms of the characterization? And, of course, how does all this relate to the risk and the associated benefits of these products?

This is not meant to be a complete list. The rest of the speakers this morning will expand on this much more completely.

But we have more than just the molecular complexity. We have the process complexity, and I would like to spend a few moments on that.

The process complexity, biological versus chemical processes and, in fact, some of the processes we talk about are mixtures of both. That complexity is generated by the number of steps, by the amount of time taken within those steps, by the reagents, by the conditions, and when we look at this, from some perspective, it can appear overwhelming, because when you think about the large experimental, the large operating space that

this complexity generates, one wonders how we could be as far as we are today sometimes.

If we do a self-assessment and we ask, well, when we introduce a process to make a biotherapeutic product, do we know what those optimum conditions are for quantity and quality at the point that we launch a product? No, we don't; and if you waited til then, we wouldn't be launching.

Do, under routine manufacturing conditions, we continue to improve the quantity and the quality of the product being made? Sure. I think it is safe to say that with the probably 100 or more products that I have been associated with over my career, I have seen these improvements time and time again.

Well, let's take an example of a process for a simple molecule, one, I think, which we can all agree is very well characterized, and that is penicillin.

Now, I'm accused for having small print on this slide, for several reasons. One is that it

makes the exam that you are going to get a little more complex. The other is it doesn't really matter that you read the small print, but that you get a sense of the overall process.

When we look at the penicillin, the flow along the top line is the biological route for making penicillin by fermentation, and flow along the bottom line, in green, is the route for recovery and purification of that final product.

A biological process, many steps, many reagents, well practiced for many years.

If we look at a biological process for a monoclonal antibody, it is a bit more complex. Along the top line, in green, is still the synthetic step, fermentation, and then, in subsequent steps, in blue and in red, what we see is a somewhat more complex, but, again, sequential unit operations for the recovery and purification of the product.

So the design and operation of this space creates some complexity, yet, in both cases, we do have processes that are biologically driven, and

the protocols are not at all that different.

So one of the lessons we learn from such comparisons is that it is not biological manufacturing, per se, that adds the uncertainty, because certainly there is little uncertainty with production of penicillin.

There is more with the production of more complex molecules. Of course, it is our understanding of how we can relate those manufacturing operations to the product itself and the barriers in that understanding are in our analytical capabilities and the ability to completely explore that very large operating experimental space generated by those many different unit operations.

Of course, the implications of being able to understand those relationships is important to both improving the existing processes, as well as developing the new processes, because in all cases, when we begin, we are not at the optimum; but as we go forward and as we generate knowledge, we are

doing a better job of it and we are learning to improve both the new, as well as the old.

Well, when we probe the process complexity to reduce uncertainty, what are the strategies that we have used to do it over the years? Well, the idea that you operate under some set of validated conditions, meaning that you can reproduce something that is inherently uncertain three times or more.

Inherently, in these processes there is variance and one of the things that, frankly, I don't think we have done a good job of is to listen to that variance and to learn from it.

We don't always do things the same way, whether it's a chemical process or a biological process. We don't always do things the same way, but we can learn from the way we do it each time, if we put the analytics in place and if we listen to the data and if we create that knowledge, and it is important to expand that knowledge in that broad operating space.

The FDA initiative on process sound

ecotechnologies is about using analytical tools to improve how we understand the underlying science around these processes, and, of course, this constantly gives us the opportunity to relate quality of the product and quality of the process at the same time.

To take just a couple of quick slides, from a process perspective, the supply of oxygen to a process is very important. As we go from small reactors, on the far left-hand side, in the microliter scale, to the cubic meter scale on the right-hand side, we want to know how does oxygen affect the cell and how does it respond.

In work we have done with alpha-1 antitrypsin, we were concerned about oxidation of ethionine, particularly at 358 and 351, and if you treat alpha-1 antitrypsin with oxygen, you will oxidize them and you can inactivate them, but with very good analytical technology, you can show, quite unequivocally, that under biological conditions, those oxidations don't occur, even though they may occur under subsequent process

conditions.

We can measure, we know where we are, we know what the relationship of the oxidation of those states is to the quality of the product.

One thing we did find is that there was an oxygen-dependent proteolytic degradation, but pursuing the background proteases in the cell by the elimination of the CLIPP system, one can eliminate that oxygen-dependent proteolytic degradation.

So as a consequence of looking at where we are, having the right metrics, being able to go in and make these changes, one can, in a very rational way, begin to probe the process with the same sophistication that we probe the cell.

Do we have analytical techniques that you can apply at the cellular level? Well, at one extreme, we can apply microarray experiments. A daunting thought. Do we want all those 4,000 genes and e. Coli to be the same from day to day? No, I don't think we do.

Do we want to know how the pathways,

which, fortunately, there are only about 200 pathways in e. Coli, do we want to know how they behave? Well, that might be interesting.

So if we begin to ask questions like what is the effect of oxygen, one can show that, in the case of e. Coli, if you go from air to oxygen, there is a short-term response from the OxyIR regulon, whereas when you do the same experiment, the long-term response is from the SOCS RS regulon.

So we can begin to probe cells. We can ask experiments, we can ask a cell how it is doing, and we can begin to create a level of knowledge that defines where the risk is, where the uncertainty is, strategies for overcoming it, eliminating it, and it's not simply a black box.

But as one looks at manufacturing, it's not about just the cell. It's not about just the materials that are there.

One of the things that we have learned, from a large body of experience in making these kind of products, is that the product is about the materials we use, it's about the conditions, the

configuration of the operation, and the package we put it in.

So when we think about the questions that we ask and the metrics that we put in place, it needs to recognize that it is in a system, illustrated here.

Well, this system also allows us and this strategy also allows us to think about the process in terms of a manufacturing science.

The area where I think we have excelled in the industry and in academia is in the product science, in the analytical techniques, defining and characterizing that product.

As we look at the intensity of process understanding, as we move up that scale, the goal is to take what is now largely descriptive knowledge, move it up into correlative knowledge, and move through mechanistic knowledge into first principles.

Are we there when it comes to processes? Largely speaking, no. Are we going to be there in a short period of time when it comes to processes?

Largely speaking, no.

There's a lot going on. Does it matter? I don't think so. But do we know which aspects of the process, which aspects that we have to measure are going to be important? There is where I think we have made good progress and when we look at where we are going, I think we can see that there is the opportunity to intensify our process understanding, to move towards a higher level through appropriate analytical techniques, and move from the minimum that we use today to move up in that scale, and that is going to reduce the uncertainty associated with the process complexity.

When we look at the processes, again, let's go back and look at where we are today, and we see a continuum. If you will work with me through this table, there are four things that we can talk about in terms of change in follow-on biologics.

There is the product, the process, the location, and the company, A, B, C, D.

If we look at existing processes, what we

talk about is keeping the same product, improving the process to be one, and we have become generally pretty comfortable with how well we do that.

When we have a good process, but, for market reasons or security reasons or capacity reasons or economic reasons, we decide that we want to have a second location or move the location of a process, we keep the same product, we keep our improved process, because we have learned that that is better, and we move to a new location, and we have learned to do that reasonably well, also.

Then as we go down this line, we now take that same product and we may have a completely new process. Certainly, as you go to making a recombinant molecule with a completely different host and a completely different recovery protocol, that would be a new process and we might even call that product a prime, and it may be in the same location, by the same company.

Then as we go further down this spectrum, we see that we may, again, be going by a different process to make a similar product, a prime. The

process may be, in this case, the same as B2, but it may be in a new location and by a new company.

So that as we look at these four areas where we consider change, there is a spectrum, and, of course, our challenge is to identify where we want to go and how we are going to measure where we are relative to this spectrum.

So what are the next steps? Where do we go from here? Where do we need agreement? I think we need agreement that follow-on biologics are a reality today. We need agreement on the nomenclature, so that we can have a common dialogue and not be approaching the elephant, as I illustrated in the earlier slide.

We need agreement on the analytical methods to measure where we are. We need a discussion around the reference standards and what those reference standards might mean, because they are going to be different in this world than they are some others.

Understanding what is acceptable uncertainty, because the only way to absolutely

minimize uncertainty is not to do it, not to go on the mountain. And we need a strategy for managing this in a continuum of risk.

So I think the challenge is to certainly create clarity of the nomenclature, and a lot of progress has been made, to articulate those scientific principles that will help us resolve where the uncertainty is and to relate these principles to this continuum.

So in closing, we are at a very exciting time, I think, in the industry and in the science. There are significant incentives to build on for many within this world.

It is important to get it right, because failure to get it right by either the innovators or the followers will hurt all the players, not the least of which is the patient.

Good luck in the next few days.

[Applause.]

MR. WEBBER: That was a great talk. I think it really sort of sets the stage, in one way,

to show that manufacture of quality pharmaceuticals is not a trivial task, either for the innovator companies or those who follow behind.

With keeping on schedule, I think what I will do is jump right to Dr. Kozlowski's talk.

Now, there have been a number of iterations of the agenda on the DIA website, and, earlier on, there was discussion of the white paper and we had talked previously about the white paper, that would give a background discussion of FDA policy in this area.

That paper is still under preparation. Unfortunately, we weren't able to get it out prior to this meeting, but I think Steve has put together a very good talk even so, without having that in hand, and I think it will be quite interesting to hear what he has to say.

So I certainly welcome Steve.

DR. KOZLOWSKI: Thank you very much. Last week, in the Washington Post, there was an article on follow-on proteins and trying to present some of the issues about it, and I think one question that

was phrased in the article was can generic manufacturers make biologic products, and that is not necessarily really the question.

The question is can anybody compare products that are biologic or complex in nature to be able to make follow-ons. So I would like to talk a little bit about some of the background and concepts that have gone into thinking about this and how they have played out in the agency so far.

First of all, I want to talk a little bit about history. Clearly, as Dr. Cooney mentioned, there are examples of multisource products, and the agency has a history of how it has dealt with these products.

Then I would like to talk a little bit about protein structure and how one can look at protein structure in terms of follow-ons.

I would like to talk about, once one thinks about the issues in terms of structure, what parameters, algorithms and outcomes are possible for looking at this; then to talk a little bit about complexity and risk; and then, finally, to

talk a little bit about the use of examples and rules of engagement at the conference.

So to start off with history. There are a number of regulatory statutes that affect proteins. Clearly, the PHS Act and some wording from the PHS Act is that we approve on the basis that the subject of the application is safe, pure, and potent.

The Federal Food, Drug and Cosmetic Act is the other statute for which proteins are regulated, and there is more detail in terms of how to approve there. There are different levels of approval; 505(b)(1), which requires full reports of safety and efficacy, and the applicant must either have control or the right of reference to all the data for this full support.

There is 505(b)(2), in which all that same data must exist; however, the applicant is referring to data that is not really under their control and making the case that all that data is there.

Then, finally, 505(j), which depends on

the fact that a product is a duplicate of another product.

Now, the PHS Act, because it doesn't quite have the detail, can be interpreted to have greater flexibility. Obviously, there has been debate about that and I am not making a legal statement, but, clearly, the way the PHS Act is stated suggests there might be a variety of ways to look for safety, purity and potency.

Furthermore, there is more complexity, because in 1962, efficacy was added as a requirement for drug approval, and, therefore, the agency had to deal with a variety of products which were approved based on safety and not on efficacy and how to deal with them, and there was a DESI process, a drug efficacy study implementation process, to try and look at these products and figure out how to show retroactively that they had efficacy.

So in looking at these rules, how do they relate to the question of follow-on? So the first thing I would like to talk about a little bit is

generics.

Clearly, in 1984, the Hatch-Waxman bill brought forward the concept of generics, which was translated both into the Food, Drug and Cosmetic Act and into the CFR regulations.

There is also potentially a way of referring to generics before that. Clearly, with the DESI products, there was a question of DESI similars and once they were brought in the line of efficacy, could one DESI-like product be shown to be similar to another.

So generic-like things may have happened somewhat earlier than 1984.

Since these rules apply only to some proteins, and some proteins are under the PHS Act, I am referring to this not for a legal basis, but the scientific principles that were involved in the Hatch-Waxman generics, presumably, should apply to all products, but not necessarily the legal implications.

So, first of all, there are pharmaceutical equivalents, which states that if a product has the

same active ingredients, dosage, form, route, and strength, that is one component of making a generic, and then there's bioequivalence, in which that same active pharmaceutical ingredient has the same rate and extent of absorbance and availability to the site of action.

So these two things together create a state which is referred to as therapeutic equivalents. This therapeutic equivalent is the basis for these 505(j) approvals. They need both pharmaceutical and bioequivalence, and, generally, should not have additional clinical or certain types of pre-clinical studies beyond bioequivalence in order to have this status.

Now, therapeutic equivalence implies interchangeability, which is a key concept; that a pharmacist can switch a brand name, unless instructed not to do so.

Now, this question of interchangeability is not only limited to 505(j) type approvals. Clearly, it meets therapeutic equivalence, but it can be shown in other ways.

So under 505(b), for instance, thyroid hormone is a product that has approval under (b)(2), which has an A/B rating, which is one form

of interchangeability ratings, there are others. That is a pre-1962 drug and just to show that it happens afterwards, lisinopril is a product after 1962 which was not approved under (j) and has an A/B rating.

To move on to a little bit of examples of products under the PHS Act. So there are a variety of natural source products regulated under the PHS Act. Many of these have required full clinical studies, but there is a subset of these where the clinical studies are clearly a lot less than what ICH guidelines would recommend as full numbers, and these include albumins, IVIG, allergenic products.

The limited clinical data required for some of these products is based on very well understood standardized processes which have existed for a very long time, such as the co-infractionation and some form of extractions.

There is a great deal of experience with

the indications and there's probably a limited number of patients in some of these indications, and there is a great deal of experience with these products.

So even for natural source products under the PHS Act, not all the clinical requirements for products have been the same as initially or as now.

Regarding recombinant products and monoclonal antibodies, there is a long history now of changes by single manufacturers in process, some of them minor, some of them extensive, and some of these have required additional clinical studies and some of them have not.

Furthermore, there is improved understanding of the pharmacology of certain of our products, and, clearly, the requirements for products, once a good biomarker has been established or other knowledge has been increased, may not be the same as for the initial product.

Moving on to the Food, Drug and Cosmetic Act products before 1962. So we have some nonglycosylated low molecular weight examples.

Insulin, although there's a lot of multi-source product insulin, at the present time, they have all been approved under the full pathway.

Glucagon, though, a similar size protein, does have a recombinant that was approved under the 505(b)(2).

There are complex mixtures regulated under the Food, Drug and Cosmetic Act, and pancreatic enzymes, which there is now an effort through a draft guidance and a variety of strategies to bring these products under NDA, and the first NDA for them was in 1996.

All these products have had a long history of use, clearly, they were all from before 1962, and a large amount of clinical understanding based on that long history.

If we look at some products that are post-1962, nonglycosylated proteins, we have human growth hormone, which, again, there are multi-source human growth hormones, but at least in the United States, they have all been the full 505(b)(1) pathway to date.

We have some small proteins that have been brought up for some abbreviated approval, but various clinical issues about them, such as the potential for hypersensitivity, have limited that pathway. So not everything is the same.

For glycosylated proteins, we have FSH,

for which all of them have been the full pathway to date. However, the mixture of FSH and LH menotropin had a 505(j) approval in 1997. It was actually never distributed under that approval, but it was approved.

Since then, there have been a number of approvals through the 505(b)(2) pathway.

So far, throughout all the experience of the agency, the only protein ever approved under the (j) type pathway was manitropin.

Now, all this has happened over a large period of time, and I would like to put this into somewhat of a historical context with the set of time lines.

Now, before I even start showing these dates, some of these dates can be challenged.

There may be examples on one side of the bar or the other that I have. I'm not guaranteeing exact accuracy, but I think this time line shows some of the general gist of changes over time.

So to start with recombinant DNA technology. So in 1976, we had the NIH guidelines on recombinant DNA work. There was a lot of fear associated with the recombinant DNA and bacteria leaking out into the environment and the horrendous potential complications of this.

The following year, there were more than a dozen bills in Congress to limit recombinant DNA technology; again, the idea that this is a very scary and dangerous step.

In 1982, we had the first recombinant product, recombinant insulin, followed shortly thereafter by growth hormone.

In 1986, there was a Federal Register Notice that no recombinant DNA proteins could be approved under the ANDA or (j) pathway. Again, maybe a lot of reasons for this, but one of them, potentially, is the fear that recombinant DNA

products are somehow a different spectrum than other products.

Again, we have recombinant EPO in the late '80s, and in the 1990s, we have Jurassic Park, which is another example of how primal fear, maybe not just of dinosaurs, but, also, of the potential danger of recombinant DNA once again rears its head.

In 1993, the FDA issued a statement that genetically-engineered food is safe. Shortly thereafter, we had the first recombinant tomato. I think it made the cover of Science Magazine.

So clearly, although the fear has always been an issue in recombinant DNA technology, it has been alleviated. Since then, there are scores of recombinant DNA products that have been approved and have a history of safe and effective use.

One of the next attributes that has changed over time is our ability to characterize things, structural characterization.

So in 1982, with plasma desorption mass spec, things about a 1,000 molecular weight could

be measured at nanomole amounts.

In 1986, we were suddenly able to look at three-dimensional structure by a method other than crystallography, using nuclear Oberhauser effect and NMR. Within ten years of the previous time line mark on mass spec, with electrospray and other methods of looking through mass spec, suddenly we were measuring proteins greater than a 100,000 molecular weight. So that is a million-fold increase in sensitivity.

People thought that the increase in computers is the unheralded greatest increase in terms of capability, but I would say a million-fold increase over ten years is a pretty reasonable approximation of advances in computer science.

Then by the late '90s, routinely, NMR could look at 20 to 30,000 molecular weight structures, and there is certainly a lot of experimental data looking at larger molecules.

In 2002, the Nobel Prize was awarded both to advances in NMR and mass spec, showing what critical advances there were in this field, and

these are just two types of characterization. Characterization has improved across the board in other areas.

So we can see, over time, our fear of recombinants has decreased. Our ability to characterize proteins has increased tremendously.

Now, what are the consequences of this characterization? Now we can see much more heterogeneity. Does it matter or does it not matter?

So in the early '90s, there are some papers looking at erythropoietin and the fact that glycoforms can affect both PK and bio activity. Shortly thereafter, there were a lot of papers about luteinizing hormone, showing that both PK and bio activity can be affected by charge variants.

As we move through the '90s, there's a lot of papers showing that the glycoforms of monoclonal antibodies can affect their effector function.

So as we have increased our ability to characterize variants, we also are beginning to get an idea that these variants can matter in some

situations.

Finally, immunogenicity. Immunogenicity has always been an issue. Insulin has had immunogenicity. There has always been a fear of loss of efficacy from immunogenicity in products, such as Factor-8. There have been issues about hypersensitivity, and there have been some reports about products where immune response against endogenous cell proteins has occurred, such as with TPO.

However, I think the biggest public awareness of the potential risk of immunogenicity in terms of affecting, in a long-term way, endogenous proteins has been erythropoietin, and this really only was noted around the turn of the millennium.

So if we put some of our past decisions in this context, and I would like to take one, for example, menotropin, that if you look at the menotropin approval, it started sort of before a lot of the structural variants were in the public domain, and concurrent with understanding of the

effect of structural variants, and it occurred before the sense that cell proteins represented a potentially high risk and that those cell proteins' high risk, in fact, was caused by a manufacturing change, not even by a different manufacturer.

So we have a bit of this historical context. What does this say about the agency's historical decisions? How can they be looked at? So what I would say is the FDA policy has evolved over many years and continues to evolve, and it is based on analytical techniques and what they can characterize, manufacturing practices and controls, Dr. Cooney illustrated our ability to control the process, really affects what we can do, and the clinical and regulatory experience, both in general and specific to products.

Also, one cannot forget legal frameworks, because all these has to happen within a legal framework and in a way that tries to ensure consistency and fairness.

So if we take all these historical messages and put them together, what we have done

in the past, we have had changes in clinical requirements and they have occurred for related protein products, and there is no doubt about that, but they have both increased, sometimes, requirements, finding a new adverse event, or they decreased what we required, depending on what was observed.

They have occurred with natural source products and recombinant products. They have occurred under both statutes regulating products. The reductions under the Federal Food, Drug and Cosmetic Act, though, have generally been through the (b)(2) pathway, and, also, significant within manufacturer chains, have been deemed comparable, although, as I mentioned before, some of them have required clinical studies.

Now, key issues involved in making these decisions have been the clinical indication, the product class, and product knowledge, and these decisions can be tied to therapeutic index and duration and breadth of experience with a product.

So to try and tie this into a visual

scheme, this chart is presented.

Now, before even thinking about this chart, these changes are nonlinear. This is an extremely idealized representation and should not be taken in a sort of mathematical sense, but just conceptually.

So if we look at the Y axis, we have pharmacological knowledge. This is what you need to show a drug is safe and efficacious.

The total amount, whatever that is at any given time. And then you have knowledge that you might be able to transfer, and it may be transferrable simply by similarity of indication, nothing to do with looking at similarity of product.

It may be transferrable by product class, or it may be transferrable by similarity of product.

This information can then be used to reduce the burden on someone making the next product. Again, to reiterate sort of what I started with, this total line, the pharmacological

knowledge for safety and efficacy, is a moving platform and it changes based on our experience. So this is a very dynamic type of knowledge.

So I mentioned comparability within manufacturers. I would like to make a few comments on that, because I think there is a certain amount of debate about how that relates to follow-on products.

So we have the 1996 comparability guidance. We have ICH Q5E, which goes to great lengths to say it is only dealing with within manufacturer changes.

What I would like to comment on is that the role of this information and these guidances on follow-on proteins is not regulatory guidance in the strict sense, but you cannot divorce the scientific principles in these documents from the scientific questions of follow-ons. Therefore, they are on the table for those reasons.

And a point related to this, the requirements for similarity between manufacturers should certainly never be less than comparability

within the same manufacturer.

So for instance, stability is something mentioned in Q5E to assess differences in products, so that certainly should not be excluded for assessing differences between manufacturers.

Now, as Dr. Cooney mentioned, we are not alone in this. We are part of the whole world, mentioned in ICH documents. So the EMEA has issued a draft guideline on similar biological medicinal products, which was released for comment in November of 2002, and I would like to take a few bits of that, and one of them is repeating what Dr. Cooney showed, but I think it is worth reiterating.

"Due to the complexity of biological biotechnology products, a generic approach is scientifically not appropriate." There is a biosimilar approach, and it is more likely with highly purified products.

So ironically, recombinant DNA is one of the products listed, where, historically, there was great fear about it, and I would actually say, in Europe, probably the fear of food that is

recombinant is still pretty high, maybe not drugs, but that has certainly changed, and more difficult products, that are difficult to characterize, are less likely and some natural source products are mentioned in this.

The document also mentions subtle differences and that because subtle differences may always exist, in order to have pharmaco vigilance, the specific product always needs to be able to be identified, if it is a follow-on type product.

So we have talked a bit about the history. I would like to move on to protein structure and how that may relate to follow-ons.

So there is a concept of structure and function and if you look at our regulatory guidance for specifications for small molecules, it doesn't contain the term "potency," because on a batch-to-batch basis, potency is not an issue for molecules that you can really characterize well, because structure equals function. There is sort of this term "GRAS," which is generally regarded as safe, that we apply to products. I would say the

statement is "GRAT," generally regarded as true. I don't think anybody would challenge the fact that if you can completely characterize the structure, then you completely define the function, and this is the basis of Hatch-Waxman, the fact that structure and function are related.

The question is not whether this would apply to biotechnology products, if we could completely define structure, but whether or not we can, and this relates to complexity and complexity exists in all forms.

We know, from experience with conjugated estrogens and heparins and other products that are not large proteins, that complexity can be a big factor in establishing follow-on or generic type products.

But our topic is really proteins and proteins are large and they are often extremely complex. So to briefly talk about proteins, they have primary structure, they have higher order structure, they have post-translational modifications, which leads to a lot of

heterogeneity.

If you look at a Statin, molecularly at about 400 daltons, and you compare it to a monoclonal antibody which has a molecular weight of a 150,000 daltons, and even if I'm only showing a third of the monoclonal antibody in FAB, you can see the size of one dwarfs the other.

The post-translational modifications for the amount of monoclonal antibody can be larger in size than the small molecule. So you are dealing in variants for which the differences are larger than the size of many other drugs.

Now, if you look at this problem, how do you deal with difficulties in measuring structure and heterogeneity, that is not just limited to follow-on product. It's a product that manufacturers, who manufacture as innovators, have to deal with all the time.

How much of this product can we measure or see? Clearly, we use release tests for lot-to-lot measurements, but they are a small fraction of really the molecule and what we measure in it.

Then there's characterization tests which are used for measuring effects of process changes or to initially characterize a molecule, and we

assume those see a larger fraction of the molecule, but, again, I think very few people feel we see all the molecule.

So the question is what is the leftover space? Historically, we have referred to the leftover space as defined by process, that those bits of information we can't extract otherwise, we extract by process.

A critical question for follow-ons is how much are we really still extracting for process, at least for some of our molecules, and can more aggressive characterization or limited clinical studies fill in that hole.

So based on this, I would like to make some definitions in terms of the discussion.

Identical. Identical is a term, and I know that at the stakeholder meeting, there was reference to the fact that "identical" appeared in one of the FDA approvals, "identical" can mean many

things in the language that it is used for; may have meant something different then than it means now.

I would say what "identical" means now are the product attributes are the same, all of them, as the comparator product, and this is a difficult task for complex products.

Similar. The product attributes are similar enough to establish the same safety and efficacy of the comparator drug. So the attributes do not need to be identical, but the relevant ones need to be enough to show that the drugs should be behave the same way.

These determinations are all based on adequately assays and adequate control of variability, and I cannot reiterate the importance of that, and we'll put it under a black box label warning that really, unless you can truly measure these things and know that you are reliably measuring them, then you can't make the comparison.

So how do we evaluate similarity? There are two real issues. One is what do you compare

to, how do you compare to, and the second is how close is close enough.

So in terms of comparators, you need an accessible reference drug substance or active pharmaceutical ingredient. You need something to compare to.

How you get this may be a legal issue, but you need it.

You also need multiple lots. You can't compare one-to-one. We know there is so much variability within products and within assays that you need sufficient numbers to be able to make meaningful comparison.

If there are multiple source products, it may give you multiple comparators. Historically, for generics, there's generally been a tendency to have a single reference drug. However, the question of whether that necessarily needs to apply for follow-ons is one worth thinking about.

The statistics you would use are critical and what can you bracket and what can't you bracket. For instance, if you could use multiple

source products as comparators, could you bracket them? Would that lead to combinatoric problems that are difficult?

Then besides what you compare to and how you decide what you compare to, there is pharmacological knowledge; there is what you extract from that comparison; how close is close enough, and that depends on the molecular mechanism of action, structure-function relationships, and, again, if you have multiple source products on the market, not necessarily as direct comparators, you may have a real feel for how these drugs behave clinically when made by different processes.

Again, clinical experience is critical. A drug which has a known set of adverse events, that information may be important and what you need to assess a follow-on and knowing the structure correlate to that would be critical in making decisions about what is close enough.

Then, again, biomarkers may give you the tools to look at structural variation in greater detail than with a hard clinical end point.

So we have some basis, at least now, that some of the issues in protein structure and follow-ons in the comparisons. What would be

strategies? What parameters, algorithms and outcomes could one foresee for such comparisons?

First of all, structural similarity is critical, and there is a lot of biochemistry to look at this. Peptide mapping with multiple enzymes is a very powerful tool. Mass spec is a very powerful tool, and we have advanced a lot in our ability to look at oligosaccharides and their heterogeneity, and there are probably other methods which are powerful to look at the biochemistry and basic structure of a molecule.

Higher order structure is a trickier question. There are absolute methods. By absolute, I don't mean absolute that this is definitely the structure, because there is nothing that does that.

By absolute, I mean that it gives you coordinates, it gives you a picture. So we have x-ray crystallography, multi-dimensional forms of

NMR. There's also relative structure, circular dichroism, a variety of signature assays, what roles can they play.

How much of the structure do you need to know? Often, when you have a flexible part of a molecule, you may only define 95 percent of the structure or 90 percent? How much is good enough? Does it matter what section you can or can't define?

Then some structures are dynamic and flexible. What role does that play in products?

Bioactivity has often been used as a surrogate for higher order structure, but I think when one is comparing between manufacturers, unless you truly understand your mechanism, bioactivity may not be covering the critical aspects of your structure.

Moving on from structure, there is heterogeneity; not just what your structure is, but what the variants are and how do they matter.

So, again, biochemical analysis is a powerful tool, but how sensitive does it need to

be? And I'm throwing out numbers. These are numbers related to follow-ons, but numbers that the agency has used in various respects.

The orange book says a one percent strength different in products is a level that matters. The Guide to Inspections states that cleaning validation has to rule out a .1 percent cross-contamination from one product to the next when you change over.

The Q3 has a variety of levels of thresholds for amounts of impurities that may or may not matter, and, often, for biotechnology products, we have aimed for parts per million for many of the process related contaminants, but, obviously, variants would be difficult to do that for.

So before we even know what is the same, we have to know how hard you have to look and what technology exists to do that.

You also need stress conditions, because you really need to look at which variants you're not seeing right away and need to be sensitive to.

For higher order structure, again, does that need to be sensitive to variants? Maybe you can tell the structure of the main component of

your mixture that you could have a five percent variant with a different structure that you wouldn't be able to see by your method.

Does that matter? Is it case by case? What should the levels of sensitivity be?

And stability is an issue. Accelerated, realtime comparative.

And combinatorics. This is something I would like to touch on a little in the next slide. We really don't deal much with combinatorics even with our innovative products. Do they matter? But it is a vast undertaking to look at them.

So here is a monoclonal antibody and these are all common variants that I am going to talk about that have been seen in one way or another.

So say it has an interminable glutamic acid. It can have a pyroglut variant to that. So there are two possibilities for that chain of the antibody; yes or no?

Deamidation. Say there are three sites, two possibilities for that, deamidated or not. Methionine oxidation, two sites, two possibilities for that for each.

Glycation, two sites, two possibilities. The oligosaccharide, and I'm not even going to talk

about all the variants, just G1 and the other arm in G2, for which there are five variants, and then the cyolation of those can occur in five ways. C-terminal lysing can be present or absent.

You multiply all those out, you have almost 10,000 variants for half an antibody, if they are independent of each other.

If you throw in the other chain, ten-to-the-eighth. Clearly, noone ever measures even a tiny fraction of these.

Their relevance in probably 99.999 percent of the time is minuscule, but we don't fully know that.

For instance, we have examples of comparability where there has been a change and nobody has figured out the cause for that change.

There is no single attribute that matters.

How do we know that that changes into a different combinatoric?

So, again, I think within manufacturers, we don't look at this. So it's not necessarily fair to say this should suddenly become an issue for follow-ons. However, a good question is how much of this is controlled by process? How much of the lack of independence that we assume for a lot of these changes are process related and how many are not?

So, again, to move on to a potential algorithm. So we've talked about structure and heterogeneity as two major issues that need to be looked at in some sort of measure of similarity, but there are also impurities.

Does the host cell matter? Do host cell impurities matter? What level of diligence needs to go into that? What levels? We talked about numbers in the previous slide. To what degree do they need to be characterized?

And formulation; clearly, we've seen

formulation interactions with complex products. Is that a new variable for formulation that doesn't exist for molecules in which there is less heterogeneity? And we have also seen container closure interactions.

So these are issues that need to be thought about in the algorithm and if we are working on a similarity approach, the answer of "yes" at any of these stages of comparison is, again, not absolute identity.

So we need some basis for making a decision about whether it's yes or no, and this is going to have to do with our prior pharmacological knowledge about the product and what matters.

And if we get "no," at what level do they mean what? I would say if, based on pharmacological knowledge, the structure is different, it's a different product.

However, for the other "nos," what is needed? I think a lot of that depends on clinical pharmacological knowledge. If one really understands a disease has good markers, it may be

that very limited studies would be able to resolve the questions based on structure.

Then, of course, a very big question that is relevant is interchangeability. What would be our criteria for that? Could it be done? How would it be measured? what would the burden of proof be for that?

So if we look at this algorithm, pharmacological knowledge enters in two ways, not one. There's basic pharmacological knowledge of the product, which is involved in the similarity decisions, and there is clinical pharmacological knowledge that helps you when the similarity doesn't quite match.

So, really, one could modify that slide I showed before and say since pharmacological knowledge goes in twice, that it's really got another dimension in this assessment of what you need for a follow-on.

So on one axis, you have similarity versus complexity; how much you really know the product is like another product. You have product

pharmacological knowledge, which includes mechanism of action, experience with multiple innovators in terms of structure, structure-function relationships, and you have clinical pharmacological knowledge, which is based on the utility of biomarkers, clinical experience, what adverse events are present, how should they be looked for, and what end points are useable.

Once you do this analysis and you decide what is necessary, the question is what are the outcomes, and there's a broad variety of potential outcomes.

Full safety and efficacy studies, ICH numbers, full requirements; some sort of modified safety, maybe targeted for a particular adverse event that is known; maybe smaller numbers.

Immunogenicity and limited toxicology may be a part of modified safety. Are these indications specific? Again, I think this might depend on the similarity of indications and what is known about them, but is there some burden for each indication to make a claim of similarity or not?

Modified efficacy. Would alternative end points and surrogates that would not be allowed for a new product be allowed for this? Would certain

biomarkers be useable? Would smaller numbers be useable? Again, bioequivalence may be a very important part of many of these, and immunogenicity, which, again, is a critical issue for these protein products.

What is the statistical basis for sides? Should they be side-by-side? How can you ensure the assays are equivalent?

So with all these issues, it is very complicated to choose an algorithm, but, again, I think that we have a lot of knowledge and there may be a path forward that is reasonable to take with all these considerations in mind.

I would like to comment a little bit on complexity and risk, because, certainly, one of the issues brought up regarding follow-on product is the risk.

To do that, I would like to borrow a slide which I have adapted from the stakeholder meeting.

This is a slide that Rob Garnick showed, and he compared a jetliner to a bicycle, and he said that is what it's like comparing biotechnology products to small molecules in terms of follow-on products.

And I would say that there is a lot in between a bicycle and a jetliner. For instance, not all bicycles are the same. Some of them probably have more rigorous specifications, if you're riding in a Tour d'France. Not all planes are the same. There are cars that lie somewhere in between, and boats, and I show this boat in trouble because I think I'd like to lead into risk.

The analogy of a jetliner and a bicycle also implies greater risk, not just greater complexity.

If you are wearing a Styrofoam helmet on a bicycle and you crash, you have an 80 percent chance of not being injured. If you're wearing a styrofoam helmet in a 747 and it goes down, I don't think it's going to give you a lot of aid.

So that analogy implies that risk is also increased in biotechnology or protein products.

I'd like to challenge that. Do complex molecules always have greater risks? I would say the answer is no, and I will give you a

hypothetical example.

You have a monoclonal antibody targeting an infectious agent. It has no reactivity with host tissues.

It is used at saturation or above. Its level of no adverse events in models is much greater than 50 times the dose in the presence of the target looking for immune complexes and other things.

You have a product like this and you compare it to a small molecule anticoagulant, it is clear that the risk of the small molecule is greater.

So that is not always true, and it's a case by case issue.

There are some shared risks, though, for proteins. Clearly, there are adventitious issue risks for a lot of the way our proteins are manufactured, but these are controllable and they

are controllable in the same way that they are controlled by innovators.

Immunogenicity is a risk and one that needs to be thought about carefully, but not always does immunogenicity lead to adverse events, and, again, one can potentially design studies to minimize or control this risk.

So moving on from complexity and risk, I would like to spend just a couple of minutes on this idea of having examples for the conference and generally how the conference should go.

So we provided some examples, and you may have handouts on this. Keith Webber mentioned them. I think they are in the framework for the discussion.

You have a single chain FV, which is considered low complexity. We have a receptor ligand which is moderate to high complexity, and we have a heterodimer with multiple active sites, which would be considered very high complexity.

These are fairly arbitrary. They are certainly not a regulatory definition of

complexity, nor necessarily even a scientific one, but I think having some sort of fenceposts with which to work off is a useful thing, and there's a lot of variations.

The single chain FV could be refolded or it could fold in its host, and that may make a difference in how one would look at it. And there are a variety of other variations. You could have a less complex structure, but a much more complicated clinical scenario, or vice versa.

The issue about changes in production hosts with these particular examples, complex formulation buffers, all of these are variables, but, again, I think having some idea that when you speak about an example, you are putting it in the context of low or high complexity will be very useful in facilitating discussion.

Some other things about facilitating discussion. Regarding specificity of comments, one should focus on principles. The discussion should generally proceed from less to more complex, and when a speaker talks, they should speak on their

view of the boundaries.

So in other words, if a person's boundary might be between glycosylated and non-glycosylated proteins that can be defined by NMR, that is where your discussion should lie, not that you can't do something more complex or a peptide is trivial.

I think that it is important to be able to focus on where the action is for a particular speaker.

If one gives an example, you know, this change happened and this occurred, without a denominator, that's not all that useful. You really need to get an idea of how many times that changes have occurred either with that manufacturer and industry and what the outcomes were, because sort of anecdotal examples, although having some value, aren't really that useful in making risk-based decisions.

Finally, I think there are important issues, but that should not have time spent on this meeting, such as adventitious agents. Even though that is a critical issue and anyone making these

products is going to need to control these through validation of clearance, appropriate use of CGMPs, it's not really a similarity question and shouldn't really be the focus of the meeting.

To move forward, there is a regulatory concept called averaging into compliance. It is inappropriate to keep on throwing in values until you get the answer you want, until it sort of averages out.

That's not a good practice for lot release and it is also not a good practice for policy. And the purpose of this meeting is not for everybody to throw in their bits and then whoever throws in more wins on the average and that is the outcome of our policy.

I think, clearly, this does not need to be an average of polarized views, but really needs to be a science-based discussion, focusing on the principles of what type of products can this be done for and how.

I would like to show a slide of credits. I would like to make the caveat that anything I

said which was problematic was me. Everything else was a number of other people who contributed to this talk.

Thank you very much for your attention.

[Applause.]

MR. WEBBER: Thanks very much, Steve. That sort of lays out the field within which we will work over the next two days.

I think we should probably just move on. I am going to introduce Blair Fraser. I don't really use these little cards very much, so I might as well put one up for him, because he will be up for the next three speakers, to really move into the plenary discussions, plenary talks on analytical, physical, and biochemical characterization of products and how that relates to the topic of follow-ons.

DR. FRASER: Thank you, Keith. Welcome to the first session, which is entitled "Approaches to Product Quality Issues: Physical, Chemical and Biological Characterization."

As proposed by Professor Cooney, part of

the purpose of this greater workshop is to examine the scientific basis for developing and producing follow-on protein products.

This session on analytical technologies will contribute to today's discussion and help us know where we are.

To that end, moving along, we will hear three speakers address selected aspects and viewpoints of the chemistry, manufacturing, and controls issues that play a role in defining the scientific basis for the assessment of follow-on protein products.

In the breakout sessions planned for this afternoon, you, the participants, may join in this dialogue.

With that said, our first speaker will be Professor William Hancock, the Bradstreet Chair of Bioanalytical Chemistry at the Barnett Institute at Northeastern University, Boston, Massachusetts.

Professor Hancock's research is directed at the study of disease mechanisms and discovery of potential therapeutic agents by proteomic analysis

of biological fluids and tissue samples.

Building upon his career in analytical and protein chemistry, Dr. Hancock has actively embraced the area of proteomics and currently serves as editor-in-chief, Journal of Proteomic Research, a new journal of the American Chemical Society.

Therefore, it should be no surprise that Dr. Hancock will present a talk entitled "The Challenge of Follow-On Biologics: The Role of Proteomics."

Dr. Hancock?

DR. HANCOCK: Thank you, Blair, for the introduction. It reminds me of a few years ago, I guess in the early '80s, where I had the good fortune to attend the first consensus-forming session on the biotechnology industry, and somewhat after, I visited Blair at the FDA and he was showing me, in a very excited way, that he had just done one of the first mass spectrometry measurements of a protein, which was insulin.

Those were exciting days and it is good to

see that we still, as a group, are having some future challenges.

So I would like to talk about the role of proteomics, and I think a very exciting role.

I would like to acknowledge members in my research group, students Yongui Chao, Yang Ziping, I can't point to them unfortunately. My collaborator, Barry Kay, at the Barnett Institute. Haven Baker, Billy Wu, in my group, as well.

Also, our clinical collaborators in the Boston area, Lindsay Harris, Darrell Palmer-Toy, Dennis Sgroi. Collaborators at ThermoFinnigan and Robert Gerszten, Harvard Medical School.

So, now, the role of proteomics as we move forward. I thought I would begin by showing three rather fuzzy black boxes. As we all know, these are going to be challenges as we move forward.

The insertion of genetic material into a cell is still a complex process and that is one of the black boxes.

Fermentation processes, we heard in the initial talks, the scale-up of fermentation

process, complex, and many things can go on.

Then, of course, we have the ultimate black box, which are the animal studies, translation into human studies, a variability of individuals.

Now, with FDA's strong support and encouragement, there are a number of initiatives, such as process analytical technology, translational medicine, that is already being applied to help this black box study.

But what about proteomics? Proteomics has a really occurred in another sphere. The sequencing of the human genome was followed by the proteome. So tools were developed for this new challenge, in a parallel track to the biotechnology industry.

So I'd like to move through some examples, and so I will just really give these as, I think, a taste of the future, as we can see what proteomics can do for the industry.

Improve characterization of the final product; raw material analysis; more global

analysis of the fermentation process; plasma proteomics, and, of course, its role in translational medicine; and then we can think about future challenges.

So on the right-hand side was probably the mass spectrometry we looked at in the early days with insulin. It was an impressive tool, but things really came along in terms of mass spectrometers for looking at proteins.

I think in the early days of proteomics, when I visited the biotechnology industry, people would often say, "Well, you know, we don't do proteomics; we do protein chemistry. We make one product and we look at it very carefully. What's all this global business?"

But what happened then with the excitement of the genome sequencing is a new powerful generation of mass spectrometers were developed to do global protein analysis, and these are now, of course, available for the industry.

So on the left-hand side, I give an example of a new hybrid mass spectrometer, a linear

ion trap, with a Furrier Transfer Mass Spectrometer, and that's one example of the new platforms.

So I'd like to just go back then to the problem and challenge of characterizing a product. It's very nice when the previous speakers help you in your presentation.

So the comment about combinatorial mixtures, we have variants of variants of variants.

One problem is when we take a protein, we chew it up into very small fragments, we don't know what fragment goes with what fragments. But that is what we had to do with the old mass spectrometers. We could only look at small peptides.

But at the institute, Barry and I have been looking at extended range proteomic analysis, where you can look at large fragments, such as generated by the rarer cutters. In the past, those fragments, which are sort of a top-down analysis, you could not characterize.

So by this online coupling of the HPLC

with this mass spectrometer, we can look at large chunks of the protein, and so we can do fragmentation and high mass accuracy.

So here is an example then of this enzyme, as I say, coupled to chromatography. It's not cheap, but then it allows you to give the sort of information that you couldn't have got with the previous generation.

So let me just give one example of the epidermal growth factor receptor, a favorite drug target. This is a collaboration with Lance Liotta and Chip Petracoin and Barry's group.

So I just show you two large peptide fragments here generated by a rare cutter, one 37 residues and one 32 residues. The point of this, two points, really, by looking at these larger peptides, and we can do even bigger ones, we get a ten to 300-fold gain in sensitivity. These peptides ionize better because of the multiple internal charge sites.

Now, we need this, because EGF has at least eight sites of phosphorylation. We found, of

them, only one in the unstimulated sample. But there are 11 sites of glycosylation, of which we found three in this study, we're now up to eight.

So what goes with what? That's the challenge. You might have the same overall composition, but different mixtures.

In a collaboration with Berlex, we have been doing some raw material analysis. As I said, I am going to jump around a bit, but the idea is proteomics can add, in several areas, extra value.

So raw material control, obviously, if it's a global manufacturer, supply coming from all over the place, not everything is going to be shipped from New Zealand.

So we did, with Erno and Mike McCaman, a study of a number of fetal bovine serum lots. I'll just show you two.

Now, the raw material analysis is complex, because I think it is true that we really can't tell a great lot from a mediocre lot. It's a bit like a wine; you have to taste it, I suppose.

So on the right-hand side, there is a lot

where the cells grow particularly well. This adult retinal pigment epithelia cells. On the left, a lot is not so good. What's the difference?

Well, gross tests of these lots suggest they're all about the same. They all have about the same amount of protein and, in general, it's not easy to distinguish the three different lots.

But if you carry out a proteomic analysis, and we characterized four or 500 proteins in this analysis, I'm just going to show you, obviously, a small set here of growth factors.

What was striking is that the lot that did much better in terms of causing growth had, surprise, a lot more growth factors and a lot more binding proteins.

So this is a rough test, but it does give us a striking picture of what constitutes a good lot. The downside, of course, is it's still a black box. A number of these growth factors and binding proteins are not really very well characterized, and we're not sure what they do, but that at least does give us a feel, at this crude

level, of a raw material characterization.

So what about the fermentation process? This was a pilot study with Juan and Alex Taylor and coworkers at GSK, to have a look at then what could we learn from proteomics in terms of scale-up of a fermentation.

So this was an E. coli study, a control strain and one in which the key enzyme, phosphogluconolactonase, in the phosphates shunt was over-expressed. So let's do a global study of this change.

So here is a typical profile of the fermentation process, and, again, this subject was nicely introduced.

So we took a number of time points here before the transition from batch to fed-batch, before and after induction, and then the late of phase T6, where things have maxed out and I think there is some cell death.

So it gave us a good range across the process. So a pretty typical, I think, fermentation process. So what do the proteomics

show us?

So what you can do, then, with this very expensive, complex mass spectrometer, they are actually pretty easy to run. It's just like UV detector. But now, instead of one protein, you can measure hundreds.

So one through nine are the proteins which change most in abundance. Number 10 was an abundant protein which didn't change very much in concentration.

We did this by peak area quantitation and one of the criteria is good retention time reproducibility.

So what we see, then, is key enzymes, such as acetyl-CoA synthetase have a major increase, several hundred-fold during this process. As I said, they do plateau out.

Key enzymes, and if you just go across, you can see, these are not 50 percent increase, two-fold increase, but 50, 100, 200-fold increases, except for the trigger factor, which is essentially not moving around very much.

So we have a number of key enzymes and, also, you notice different transport proteins, all associated with the burden of producing more

nucleic acid, more protein, more plasmid production, more product production.

Let me go to the bottom line and then I will come back to--I didn't try and squeeze on the 400 different enzymes.

But after inductions, the level of acetyl-CaA synthetase were strongly upregulated, in both studies, control and the VP upregulated.

Malate dehydrogenase and fumarase were strongly increased. So that tells us the citric acid cycle under the conditions of the fermenter were strongly upregulated.

If we over-express phosphogluconolactonase, the TC enzymes go up even much more, substantial further increase.

If you look at the details of this study, it shows we get better glucose utilization, we get increased growth rate and yield, by just engineering the single enzyme, and this results in

a much more efficient increased flow through the acetyl-CoA synthetase pathway.

And just going back to this overall figure, then, the scrolls show that one of the key needs of the cell is increased nucleotide in amino acid synthesis. The citric acid is down at the bottom and the stars show the enzymes which were the most upregulated in the study.

I could show you another hundred or so, but this is just the highlights.

So I think this is just the tip of the iceberg. Clearly, we can understand much better the physiology of the cells in the fermenter with these proteomic studies.

So now what about the big challenge? Translational medicine or understanding why particular patient sub-groups are affected by minor product changes, apparently.

So the challenge there, of course, is plasma and serum proteomics, a tremendous challenge because of the complexity of the media, but, of course, it is the diagnostic fluid.

So we have developed two new platforms to allow us to go much lower, and that has been a problem for proteomics in the past, is the

inability to see the interesting low level proteins.

One is using lectins and the other is using what we call a peptidome, and I will just give you some highlights as an idea of where proteomics is going.

Now, at the end of it all, we want a stable biomarker. So there are a number of properties which you can read here which select a marker which is stable, because for this to work, you have to be able to use it in clinical labs around the world. You cannot have a PHD collector, you cannot have exotic chemicals to stabilize the sample, but there are things that can help us.

Highly disulfide linked, highly glycosylated, proline rich, things that allow the marker to be protease resistant.

So with that in mind, we have developed a depletion strategy, which uses multiple lectins.

Lectin, being around a long time, it's like nature's antibodies to glycosylation, except they are not produced by an immune response.

So by using combinations, we can get a very high capture of glycoproteins from plasma. Of course, we can also look at the things that don't bind, the non-glycosylated proteins.

You have to get rid of albumin and some of the abundant proteins because they get in the way, but it gives you a deep depletion.

So here is the combination of ones we have developed for serum for breast cancer patients. Con A captures cyolated oligosaccharides. Wheat germ agglutinin goes for largely cyolated structure, jacalin for O-linked.

Of course, these have broad specificity, but the idea is we use combinations and then we use specific displaces, and together they work better because of co-optivity.

So it's a simple thing. You then just load your plasma sample or serum and capture, and you come to a startling observation, and Lee

Anderson has been saying this for some time, but we find in this study that over half of the plasma proteins are glycosylated.

So my point is if you are into biomarkers and plasma and you're not looking at glycosylation, what are you doing?

But, of course, we have been lacking tools in the past. And it's not just the liver that's secreting these glycoproteins.

Just one quick example from Lindsay Harris. We can go deep into serum from women, younger women with breast cancer, high risk of breast cancer, and you can see we can find the ERP2 marker, plexins, P353, P73, BRCA2 gene product, mucins, embryonic antigen. We are heading towards the day where a mass spectrometer will be used for population studies of, I believe, hundreds of biomarkers.

In this study, one through with, with ductile casinomi in situ, and, six through ten, disease had mestastasized.

I mean, this study showed 1,500 proteins.

I'm just giving you an idea of the known low level biomarkers.

Turning to the peptidome, then, Liotta and Petracoin have been pushing this new idea that not all the peptides are rapidly cleared. We have molecular sponges. We have abundant serum proteins which will bind and transport peptides, not, of course, to account for the ones that are being continuously produced, as well.

These are then signposts of disease being generated by enzymes such as metaliproteases, which can be upregulated in a number of diseases.

So we have a peptidome platform. One is LCMS, another one is to use a new platform called the Nanomate, where you have a one centimeter squared nozzle. Each nozzle then allows you to do static nanospray. So one nozzle per sample. Again, an example of the technology investment in the field of proteomics.

But this allows us to do process samples in parallel and do a single sample in a few minutes, giving you much more high throughput.

So now let's look at--we captured the glycoproteins with the lectins. What about we do a simple moleculae cut. So we cut to 10,000. So the

only thing that goes through the membranes, then, are the peptidome, as we call it.

So you can see A is Coomassie, B is silver staining. Three and four are where we have, in fact, managed to get rid of the abundant proteins and given us a view, I'm sure we have losses, but a view of the peptidome of the sample.

Less than 10K means we don't have to use trypsin. We don't digest these samples. We look at the intact peptides using our extended range approach.

I won't go through lists of data, but you get a lot of peptides this way. This is just an example of something rather weird. The 100 percent methanol fraction. There's a lot of lipophilic peptides, which are apparently being carried by proteins such as albumin, and, again, you can find some low level peptides which could be markers of interesting biological processes.

In serum, you see peptide ladders from the proteolytic cascade. So, for example, from things like fibrinogen, you can see 24 daughters from one parent peptide.

So you might say, well, who is interested in that. Well, Lindsay Harris reminds me that in

breast cancer, one of the hallmarks of the disease is a defect in the coagulation process. So even these ladders from the higher abundant proteins will have some interest.

So I think I might have even made up a little bit of time. But some conclusions, then, follow-on biologics will require investments in technology, and I can see the FDA has already been stimulating this. I think, really, even without follow-on biologics, the industry would recognize that proteomics can really help in terms of more efficient production, but, now, as we look at the challenges of follow-on biologics, this will be amplified.

So I really am very encouraged. I know this on several levels, but the industry and the

agency are ready for proteomics. The only challenge is it's not going to be easy.

So if you are used to a certain level of textbook technical expertise and challenge with genomics, proteomics, of course, is much harder. The lack of the ability to amplify low level proteins and the molecular complexity, which this audience well understands, is going to make proteomics a great challenge, but then I think the reward is commensurately higher, that you are going to get a great magnification.

It's interesting, in our early proteomic studies, we find that you get a much greater increase in differential expression. Often, in expression profiling studies, you'll see a 50 percent change, a two-fold change, we'll often see a 50-fold change, a ten-fold change at the protein level, and that's probably not surprising.

So much information will be gained. We are going to work hard.

There are some serious issues, however, that the industry and the FDA really need to work

together to sequence some of the key genomes. I still find it amazing the Chinese hamster, a variant genome, has not been sequenced.

So I talked to some of my industry colleagues and they said, "Well, we expect the government to do it." I said, "Well," and at that time, there had just been a news release, the elephant genome had been sequenced.

So, now, I don't think we're going to expressing in elephants. The reason the elephant genome got sequenced is because it was of evolutionary significance.

So I think if you want the genome to be sequenced, it's going to have to be an industry initiative. And annotation is key. The mouse genome has been sequenced, but the annotation is not there at the human database level. The bovine genome, again, needs to be of quality, I think.

I would like to thank the supporters who keep the graduate students fed, and then just have a general statement, which I think we are all in agreeance that I have given an analytical talk. I

am excited about the potential of the proeteomics field, but I also understand, even with good proteomics tools, we clearly need a strong relationship with the other sectors of the industry, particularly in terms of understanding animal and human response.

Thank you.

[Applause.]

DR. FRASER: Our next speaker will be Professor Ram Sasisekharan, Professor of Biological Engineering at the Massachusetts Institute of Technology, in Cambridge, Massachusetts.

Professor Sasisekharan's research is aimed toward understanding the mechanisms governing the extracellular regulation of cell function. His talk is entitled "Characterization: Does it matter? What and How?"

DR. SASISEKHARAN: First, I would like to thank the organizers for inviting me to be able to give you my perspective on the challenges and the opportunities with analytical characterization.

As Blair introduced, my title for this

presentation is "Characterization: Does it Matter?" Of course, it does. The question is what are the fundamental issues and how do we look at the problem.

I know there is a diverse audience here and what I am going to try doing in the next 20-odd minutes is to try to capture some of the key nuances of this in as simple terms as I possibly can and not dig myself too much into scientific details.

So the way I would like to bring you this presentation is to just give you a quick outline. First is I want to frame the problem. Obviously, the debate here is challenging in that we are addressing a complex problem, which is a complex biologic.

Second, I want to use the concepts of glycans, which is glycoprotein, which, in my view and perhaps to most of the audience here, represents one of the most complex of the examples here, to illustrate the issues around characterization and how it is possible to do that.

By doing so, I will say what are glycans, why do they matter, and why and how.

Finally, of course, I will get into

characterization with the context of glycans, talk about specific techniques, issues and challenges, and some of the newer approaches, which are truly becoming a paradigm shift, if you will, to be able to access thorough characterization.

Characterization, so what? What does it get us? It gets us to the concept of equivalence for complex biologics.

So what I will do is illustrate some of those concepts through characterization, and end with some broad summary.

Perhaps it's an MIT thing. I will be using the same elephant that Charlie did, but I'm going to dig into the details here.

The first thing is that I'm, obviously, going to get into the elephant story in the context of characterization, and of course, the debate, which we all know is rather polarized, and I'm not, of course, going to comment as to who is in the

tail end of that elephant.

So one of the key questions here is that there are several different analytical techniques that can be used to get that perspective.

There is an important point here, which was already sort of hinted earlier, which is the elephant is not a simple molecule. It is a complex heterogenous molecule.

So in many ways, you cannot do a head-to-head comparison between the various techniques or compare techniques to techniques between different products or different batches, between different batches or, for that matter, between innovators and the follow-on products.

So there is a fundamental issue of how do you look at the technique in the context of this complex biological problem?

Perhaps there is a reason for this beautiful concept with regard to using the elephant, because seeing is believing and there is a reason why these people are blind; that is, they can't see.

In many ways, we are the blind men in this room and perhaps we need to begin to talk, because we know that we can see, but there are other ways

that you can figure out the middle ground.

In many ways, that also applies to the analytical techniques, which is there are ways to look at the relationship between the techniques.

But before I come to that, I just want to illustrate that there are three main points that relate to each other in some sense with regard to demystifying this complex biologic.

As I said, measurements are independent and there are strengths and weaknesses to measurements. We know the product is complex, it is heterogenous, and polydisperse, and then when you're looking at a way to characterize it, you need to figure out a way to find out the links between the measurement and the challenge of the problem; the point that Charlie said, you need to know where you want to go. So you need to define that before being able to sort of harness these

techniques to demystify that.

Obviously, the other aspect is trying to understand how do you compare products, whether it's batch-to-batch or between the innovators and follow-on, and that's where you get to the concepts around equivalence, which I will expand on, using the analytical technique framework.

So in many ways, what I will be trying to say is how can we find relationship between these various independent measurements so that we can demystify and get a more clear picture of what this complex mixture is.

And what I hope to do is to show there is a path towards doing that through specific illustrations.

And the point about bringing pieces together is where biology is headed and the way I would like to introduce the concept is, historically, biology has been a reductionist science. You take a complex problem, simplify, simplify, to get into a simple solution.

But we now do know that it is no longer a

single cell in terms of how the genes work and make proteins and life goes on, but cells are in the context of this extensive micro environment, and biology is going towards a more integrated systems approach, where you do need to look for relationships and correlation between the various measurements in order to get a more complete picture, and, of course, several different techniques and technologies sort of enable doing that and what I am going to do is expand on some of things in more greater detail.

So the way I would like to begin is to basically talk about this complexity, if you will, of proteins using these three examples, starting from the protein point of view.

They are the four-helix bundle, long-chain family of proteins, where there is a remarkable structural similarity between these molecules for human growth hormone, GCSF and EPO.

Now, if you step back and go beyond the proteins, one of the most dramatic things is the fact that the human growth hormone has no

glycosylation. The GCSF has one O-glycosylation and the EPO has four N-glycosylation, one O-glycosylation and three N-glycosylation.

I'm just using these three proteins as a reference point. It doesn't mean that there are more complex proteins. We take Factor 8, which is in the far end of the spectrum, but this is just to illustrate some of the concepts that I want to elaborate on today.

So as I said, part of the real challenge is how do you look at the glycosylation in these proteins and what does it mean. Before I jump into that, I just, obviously, need to acknowledge the fact that glycosylation is not a new story. It has been around the block.

The way that I would like to elaborate on some of the historical points with regard to glycosylation is the fact that, historically, it was more a problem to deal with. It was a hassle. People wanted to get rid of it. They always took the reductionist approach; how can I just trim everything way or just have this just major

glycoform that can be on this protein.

We know that that is not true. Obviously, people did know that quality of protein can be influenced by the degree in differences in protein and oligosaccharide structure; glycans reviewed as a gimmish, it was real nonsense, and there were some very important lessons that we have learned in the last several years, which is we cannot view glycans as impurities or just chemicals. They are not just something to account for in that mixture, and I'm going to show you that the biology has come a long way in demystifying the roles of glycans and how it fundamentally impacts the physiological processes.

Second, we learned some tough lessons on glycosylation with regard to folding, especially with the interferon and the GMCSF, where the notion of getting rid of glycosylation led to protein aggregation, which opened the kimono, if you will, with regard to the immunogenicity.

We do know from erythropoietin, which is one of the classic examples of how you can

dramatically affect PK/PD or targeting properties by just changing some of the subtle features of glycosylation.

Well, the knowledge base has increased tremendously, that we know about, at least. Up to 35 percent of protein's weight can be glycosylated. So this is an important thing that needs to be accounted for, but not in a general way, but much more in a systematic and rational way, which is what I am going to get into.

So if I were to sort of summarize a very important lesson, it is the fact that we have to view them as a heterogenous polydispersed, high information content due to the diversity in chemical structure, hence, the concept of an ensemblist structure, in many ways, which gets reflected into the complexity of the protein.

In many ways, it has changed the central dogma of molecular biology in that, historically, the concept around the central dogma was one gene to one protein, and you could describe life, and we now know that that is not true; that the functional

diversity as a consequence of glycosylation truly gives us the genotype to phenotype access, where we can truly begin to understand how we move away from a cell into a tissue and an organ system.

Glycosylation is present at that interface, dramatically affecting topology and time, thereby influencing many of the systemic properties that we need to understand.

So if I were to use one slide to drive home the point that glycosylation truly matters, it is this. A single locus affects glycan structures in dramatic ways to give you a now phenotype, this picture speaks for itself, in two different hereditary diseases, and this captures the essence of where things are, and I guess this is a picture where seeing is believing, indeed.

If you ask the question, what is the functional consequence, glycosylation affects protein function in dramatic ways, and here is an example that I am showing where lack of glycosylation or specific alteration of glycosylation dramatically affects protein

structure, and hence the functional consequence of the protein.

So I am trying to use glycosylation as a model system to give you what the complexity associated with this whole microheterogeneity.

So what I will try to then do is bring it back with regard to characterization and get into greater details.

But before I do that, let me just, at a top level, summarize the fact that glycans are critical to clinical profile at a high level. They affect folding and stability, binding to receptors and other molecules, immunogenicity through protein folding issues, pharmacokinetics, distribution, and, therefore, contributions of glycans to clinical profile are similar in importance to amino acid sequence in protein, and, therefore, it is important for us to pay attention to this and not just document this as extra structures that hang out at proteins, because there is more to the biology.

So using glycosylation as the framework, I

want to get into protein characterization. As I said, it is the harder puzzle to crack, but it is in a spectrum, and I am going to focus on a smaller spectrum, but, broadly, you can get the sense that it can be readily extended to the other more complex systems.

So I would like to go back to the earlier slide that I said the three important issues that need to be linked in some sense. First, the issue of independent measurements. These are the various analytical techniques, techniques ranging from NMR, mass spec, enzymes, chemical, a variety of different techniques that are used to make measurements.

It is my view that it's not that we need many, many more newer and more sophisticated techniques. Of course, they will be helpful, but what is more important, to frame this problem, is to try to understand what are the ways these techniques truly relate and how do they correlate, appreciating the fact that the techniques have strengths and limitations. There's no one perfect

technique.

Now, we all agree that the heterogeneity and polydispersity, in many ways, is the challenge. It's not a simple, single molecule. It is an ensemble of molecules, and how do you define the problem, keeping that in mind.

And, finally, the so what. You're going to use that information to do the batch-to-batch variation issue that was discussed, or how do you do the follow-on, the whole notion of how do you bring it to the equivalence.

So my point is that technology needs to demystify the complexity around characterization and the complexity around equivalence issue, and I'm going to get through that through specific examples today.

So I want to boot up with regard to glycan analysis, because there are many technologies that are out there, but the question, again, is what do they address, how adequate is it, what do we really need to address, and how do we get there.

So in this case, I just started with

releasing a glycan from protein, enzymatic methods. There are issues of non-specific cleavage and cannot access certain sites.

People have already looked into variations of glycans and EPO production, as an example. As you know, one of the big challenges is the fact that this is a heterogenous mixture, you would have separation challenges, resolution challenges, and overlapping peaks.

But there are strengths to these techniques and you want to focus on the strengths and make sure that you do not get into the pitfall of the limitations, and, therefore, you bring in all kinds of techniques to get more clarity around the picture.

Just going further, away from a monosaccharin, there is something more global, like the profiling of glycans using mass spec or potentially even NMR. For instance, you can get a good picture and most of the times, you get the picture of the more abundant structures, but that is not the entire puzzle.

There are these other structures that you need to account for, because in some sense, it is true, the more you thoroughly characterize

chemically, the more you completely have a sense of what the picture is, it lends itself to the structure-function concept.

But the point is you can get important measurements here, but as I used the mass spec to show, one peak would have several tens of unique structures and deconvoluting that is important.

But the key point is you want to be able to boon on these different measurements to be able to get a more thorough picture.

So if I were to frame the question, where are we with glycan analysis, what do we really need to do so that it captures the biology, it captures the challenges, and how do we use technology to get there, here is how I like to summarize.

By and large, the current approaches are limited to high abundance species, sorry for the typo there. What is required is the fact that you need to basically get into the low abundance

species, the associated glycoforms, sites of glycosylation, accurately quantifying each glycoform, analyzing subtle modification of fusocylation and sulfation, and determining sensitivity of glycoform structures to both process conditions and protein structure.

The point I want to reiterate is when you look at a protein, you want to know what are all the sugars, on what sites, how much they are, what the sequence is, and what the minor modification is, and you can imagine that an erythropoietin can have tens and hundreds of structures just looking at the glycosylation itself, and the question is how do we demystify that.

I'm going to, again, come back to the elephant analogy, to give you a sense of how complex data can really be viewed and correlation and important parameterization is possible, where you can relate these pieces of information.

Here is the elephant. Here are the various analytical techniques. You take the different measurements. Measurements have

strengths and weaknesses. What you want is to be able to mathematically find relationship, quantitative relationship, not qualitative relationship.

We are very used to seeing two chromatograms and comparing them visually. Just because they appear visually similar doesn't mean quantitatively and mathematically they are the same.

The mathematical space is different. So how do we start demystifying that picture, where you start taking meaningful measurements, looking at the relationship between these measurements, the simple analogies, how do the data start to talk to each other.

So that you can take eight different measurements, this is purely an illustration just to illustrate and get the concept out, eight different measurements, you go to 40 different measurements or a 100 different measurements, so that you start to get the various pieces coming together a puzzle.

So what has happened in this International Consortium of Functional Glycomics, where the field of glycobiology has really brought all the various

resources together from the molecule to the mouse to the humans, in terms of analytical techniques all the way to biology, is that we have taken such an approach to get genomic data, glycomics data, proteomic data from all the different targets that we have, from cells, tissues, mouse, and brought them together with a platform to integrate them, to be able to look at how various things correlate and give you an outcome and use the molecule page as a data dissemination port.

It's like a Yahoo portal site that gives you all the features associated with that. So this is doable. The question is it is important to identify what are the various pieces that need to be brought together, and, obviously, this is going to be molecule-specific.

What I am going to do now is to give you a specific example to just walk you through how that is possible, so that, at the end of the day, it is

possible to bring these different constraints and start looking at a complex mixture in the context of a space.

And how do you do that? Just an illustration of using alphabets here. You can use various enzymes or chemicals to digest the different pieces. So you could do this in a combinatorial way, quantitate the various measurements. You could then use mass spectrometry to get different masses and different kinds of information, depending on which mass spec you use.

You could use NMR to get linkage information. Bear in mind, what I am trying to do here is focus on the strength of these techniques and really make all these multiple measurements to be able to use them as constraints.

What we did was take a mathematical approach or a number-based approach, as against a character based approach. If I were to just dumb it down, it's the analogy of saying if you can take numbers, then you can use the power of computers to add every data as a byte in a computer and use

binary operations of on and off to be able to pool pieces together, you use characters like ATGC for DNA and P for protein.

So, again, it's a visual representation as against a mathematical representation, because the complexity demands or requires you to take the problem and factor in all the various pieces that truly need to come together to quantitatively describe this space.

So what this gets us is bring the pieces--the constraints together to get to your solution. So what does this do in the case of glycoprotein and what we're talking about?

In many ways, and I use this as an illustration, it gets you all the structures of the O-glycan at a particular site, the sequence and their abundance, all the different N-glycans and, particularly, the distribution and thereabouts. We can look into unusual modifications for particular sites, and we can start doing comparison where you could look for changes as a function of protein properties or process conditions or formulation

issues, so that you have a more complete picture of what is going on, and that is really needed to be able to demystify this complex mixture.

So I just want to quickly move on to say that several of the techniques are readily available for proteins and similar strategy, going all the way from protein structure analysis into the biochemical, chemical, to potentially the biological property, can be brought together to get a more complete picture of what is really needed.

So what I am now going to quickly do is to take the characterization to the next level, saying how could we use this information in order to understand, at the end of the day, if two molecules of complex mixtures are similar or identical or however you want to define it, and the point I want to introduce is concept called equivalence window for complex biologics.

In other words, it's not a point-to-point comparison, but it's the space-to-space or a box-to-box comparison. Keep in mind, it's not a visual space, it's a mathematic quantitative space.

So that there's a rigor associated with the way you can look at this.

So by definition, biologics are a heterogenist mixture and vary batch-to-batch. So if I were to use the simple analogy of, let's say, ten people in the room, six of them with blue eyes and four of them with green eyes. A very important take-home is you cannot average data, to a point that was made earlier.

In other words, it takes away from reality, because if you average this, it's basically saying, on the average, people in the room have aqua eyes, which is not the reality.

So you want to be able to capture those six blue people and four green people and try to understand what are the properties associate with that.

I just used eye as an example. There's a lot more to that, but that illustrates the concept.

So when you know that here are the people in that room, you can start using your analytical techniques, develop what we call a quantitative

relationship between these various people through those measurements, and then once it becomes quantitative, then you can start developing a mathematical window that can say by definition, here is this fuzzy complex mixture, and how does this vary from a batch-to-batch or how an innovator material can potentially be in the same window or the follow-on product could be in the same window or not.

So it becomes a very powerful tool to be able to determine the whole concept around equivalence.

So in quick summary, trying to bring together the characterization and equivalence, it is important to recognize that analytical techniques provide a perspective or projections. They have strengths and limitations.

For complex biologics, you need multiple perspectives. You need multiple constraints that truly can be brought together to demystify the characterization part, and due to inherent heterogeneity, you cannot do a point-to-point

comparison. You need to define a characterization space that is a multi-dimensional window which involves these quantitative measurements, and, therefore, you can then start to look at concepts around equivalence.

So I would like to quickly summarize, basically, with the following messages. I spoke to you about glycans as among the most complex, the complexity associate with proteins. They play a critical role in the biology and chemistry of proteins, thorough characterization of sugars are important, similar to that of protein, and, therefore, are critical to be done.

Technology makes it possible for thorough characterization and the sense of looking at this complex mixture provides a paradigm for analyzing not just sugars and proteins, it goes beyond proteins, in general, for a new way of thinking of such a complex problem.

Thank you very much.

[Applause.]

DR. FRASER: The last speaker in this

session will be Dr. Joerg Windisch, the Global Head of Technical Development, Biopharmaceuticals at Novartis.

In this role, Joerg is responsible for drug substance and drug product technical development and clinical manufacturing of all biopharmaceuticals of the Novartis Group, including both innovative and follow-on products.

Joerg holds a Ph.D. in biochemistry and has more than ten years of experience in pharmaceutical proteins and glycoproteins.

Dr. Windisch's talk is entitled "The Value of State-of-the-Art Analytical Characterization in the Development and Evaluation of Follow-On Protein Products."

Joerg?

DR. WINDISCH: Thank you very much. I would like to start with thanking the Planning Committee for giving me the opportunity to speak today on this complex and controversial and yet very exciting topic.

As was already stated, I would like to

talk about the value of analytical characterization, but also of the limitations, and there clearly are limitations.

I would like to start out with spending a little bit of time on where I think we are today, and I think the technologies to manufacture and characterize protein products have continued to evolve and have progressed rapidly, and these modern techniques enable quicker and more predictable and less costly development and regulatory decision-making, that's very important, and allowed industry to avoid unnecessary and unethical duplication of trials, and I think this is really what we are here for today, to see what the scientific basis for this is.

When you take this, then you have to, of course, look at the regulatory requirements and how they need to evolve in line with these developments of these state-of-the-art analytical techniques.

In any case, rigorous scientific criteria, and this is about science and not about regulations here today, meeting the highest standards must be

applied consistently to both original and follow-on products.

The patient doesn't care, the doctor doesn't care; they have to be safe and efficacious, in any case. If you do this right, if you choose the appropriate strategy and if you do the science correctly, with the belief that follow-on products that are as safe and efficacious as the original product can be developed.

Now, if you look back into history, of course, in 1998, recombinant biopharmaceuticals were poorly understood and their quality depended mainly on the manufacturing process and once you had demonstrated their safety and efficacy, usually in extensive clinical trials, you better did not touch that process anymore.

In the 1990s, major advances in analytical science and technologies were made, and this led to the recognition that actually there is such a thing as well characterized biopharmaceuticals.

At present day, the analytical tools allow in-depth investigation of all relevant properties

of a protein or a glycoprotein, including all of the structural elements, and, also, and we heard a lot about this already in the last speech, the post-translational modifications, especially the glycans.

Now, what are we really talking about and how does this help us with follow-on proteins? Follow-on proteins are subsequent copies, not column versions, because they should be copies of already marketed recombinant DNA-derived protein products.

They have the same mode of action and are used in the same indications as the originator product, and what they can do, what these state-of-the-art analytical techniques can do is they can provide for a better informed and more robust understanding of what is under consideration when the agency and industry discuss follow-on proteins.

Now, the next point, in my view, is a very important one, because there often is the notion that follow-on proteins depend mainly on the

comparison to an originator product, but actually that is not true, because 80 percent or so of what you have you have actually done in-house.

You have generated an extensive and sound set of data in-house, including all of the technical development, CMC part, drug substance and product development, so you do have knowledge of your process.

You have done bioassays and you will have done preclinical and clinical studies, and only on top of that you will have the comparator studies within the originator product at all relevant stages.

So if you look at that slide, you see the very broad basis of standalone product and process development, and then you have physicochemical studies, biological studies, preclinical and clinical studies, and they, of course, also provide information on your product and, in addition, they provide the comparison to the originator product.

So I think we have to be clear about what we're really talking about here.

Now, when you go into developing a follow-on protein, you have to acknowledge that they are not all the same, that there are different

levels of complexity along different parameters.

You can talk about size and mass. You can talk about folding, disulfide bridging, whether we have dimers, hetero or homodimers, oligomers, whatever, and, of course, glycosylation and other post-translational modifications, at all of those levels of complexity you need to look.

And your choice of methods and extent of analytical studies must match this complexity and, therefore, you can achieve thorough characterization.

The ability to thoroughly characterize protein products will continue to increase in time as science advances, that's clear, and this will enable evermore extensive comparisons to the reference product, and it might also allow us to venture into more complex products that are not accessible at this point.

The extent of the studies that you have to

do then at the subsequent levels, that is, the preclinical and clinical level, I'm not going to talk about those here, will then be determined case by case based on the level of understanding you have gained on the physicochemical level and the biological level, and, of course, always have to be discussed with the agency.

Now, I would like to briefly go into the value and the limitations of those comparator studies, and I think that goes back to the pyramid I just showed a minute ago.

I'd like to start out with the limitations. Comparator studies with the original product cannot substitute for full CMC development, as I have mentioned before, you'll have to do that, not substitute for physical-chemical characterization, full biological characterization, full release testing, basic nonclinical testing, case by case, and, also, clinical trials.

Comparator studies, however, may allow the follow-on sponsor to limit some of the preclinical testing, to limit those ranging studies, as they

have already been done with the originator product, limit the populations and the clinical phase three trials, and possibly, based on sound science, extrapolate the broader indications based on sound scientific rationales.

In any case, it must be science and data-driven and it will be different from product to product.

Now, into the details a little bit. When you do a study, you have to first look at the molecule, how complex it is, but the good news is you don't have to start from zero. You can use the experience gained with the innovator product over many years as a foundation for characterization and development plans.

Of course, you will not have all of this information available to you, as some of this is confidential and only available to the originator.

You have to consider state-of-the-art analytical techniques. You cannot say I'm going to use the technology that was available 15 years ago when the original product was developed, and you

have to consider relevance of quality parameters for clinical safety and efficacy, and this will ultimately lead you to the discrimination between product-related substances and impurities. I will get back to that in a minute.

I hope you can still see what is there, anyhow. Which parameters do you have to look at when you look at establishing comparability? First, on identity and purity; you have to look at the primary structure, including disulfide bridging. There's a number of different tests available, including amino acid sequence, peptide mapping with UV and MS detection, MS-MS, and, also, MS and, if you have an ion trap available, and by that you can resolve species as mistranslations, translational modifications; if you have a multi-copy expression system, also, mutations that you might have somewhere there.

Higher order structure is the next level, and here we have to be clear we are not trying to establish the structure for the first time. We were talking already earlier today about relative

structure determination and this is the main thing here, and that's why found circular dichroism and one-dimensional NMR here, because they allow a fairly easy interpretation of comparability and similarity, comparing an original and a follow-on product.

As we have heard, bioassays, I clearly agree with Dr. Kozlowski that there are limitations to this.

Mass, of course, your different types of modern mass spectrometry, and the size as opposed to mass. It's not always the same. Remember, we have three-dimensional and four-dimensional structures. Good old SDS, size exclusion chromatography, and some of the more modern methods of analytical ultrasound, field flow fractionation, dynamic and static light scattering, I will get back to those in a minute.

Charge, of course, different methods there. Capillary electrophoresis, certainly one of the more sophisticated methods there. You can look at charge variants, including deamidation,

modifications and others. Hydrophobicity, reverse phase chromatography I think is still one of the most powerful tools in protein analytics.

You can look at oxidized variants, covariant modifications. We'll also usually see the deamidated variants there. Of course, binding, that is both binding to antibodies or to receptors, immunological bindings and Western blots or ELISAs, and, of course, binding to receptors, for example, in-surface resonance.

I will not touch on the glycans too much anymore after the last presentation. I think this has been covered in great detail. So I can go through this.

So what are really the advances that we have seen over the last few years in analytical science and technology? It's not as much that we have seen so many new separation or analytical principles. It's actually the advances we have made in the existing principles.

Of course, there are new principles that really help us, but the most important thing is

really the advances we have made in the existing ones.

If you look at HPLC now, we have increased resolving power, improved position, increased sensitivity, and, of course, we can connect it to MS. Same is true if you look at MS. You've got SEMS, multi-MS. You can look at intact biomolecules at their primary structure, but, also, at their glycan structures, as we have just heard.

You can do this by having different ionization modes and mass analyzers, MS, MS and MSN, as I have said, which is really nice if you work with an ion trap, and that evaluation is also very important. I don't know if we're going to hear more about this, but the software tools available are probably just as important as the techniques themselves in order to get us ahead here.

This allows you structural characterization of both the main structures and, also, the product variants.

Capillary electrophoresis I have already

mentioned, with very high separation performance and sensitivity, and even allowing quantitation these days. NMR I think we have covered, and I will get back to those methods for aggregates in a minute anyhow.

Now, an important point is how do you actually decide whether you are close enough or not? I think that's going to be one of the main topics of this workshop.

So what are the acceptance criteria for the comparison? First of all, your main component, and don't confuse this with the impurities, has to be indistinguishable to the originator product with regard to the primary structure, to the higher order structure, size and mass, charge, hydrophobicity, and immunological bindings, and probably half a dozen more.

It is more difficult if you look at the product-related substances and impurities, how do you go about these. It must be said clearly that subtle differences in the quantitative composition of the product-related substances impurities are

expected and will be observed.

The question is do they matter, and I think here you have to look at this actually not only at two levels, but at four levels, but I've only got two of them on my slide.

First, you need to look at the originator product and which amounts of variants and which kind of batch-to-batch variability you see there. So that sets your limits.

If you manage to stay completely within that frame, you are in pretty good shape. Also, you have to look at what kinds of product variants you have there.

Is it product-related substances which are identical to the main product regarding safety and efficacy, or is it impurities which have properties that are either not known, because not investigated, or are different from the main product, and you clearly will have a little more flexibility the more you know, that is, with the product-related substances.

There's two more levels, of course. If

you cannot cover everything, and usually you will not be able to cover everything, then you have to go into your preclinical and clinical studies and show that it is still safe and efficacious.

Now, I think this is pretty much in the direction of the previous speaker. Many analytical tools are available which resolve those subtle differences in physical, chemical and biological parameters, but no single method can completely characterize all aspects of a protein.

However, how can this be achieved? This can be achieved by the scientifically sound selection of orthogonal parameters, and, by that, you can expand the analytical window and you can reduce the risk that you have blind areas, that you don't pick something up in those areas that you would not have sufficient knowledge of the product.

If you do that correctly, you can actually derive a pretty complete picture of the product.

Some special considerations. Again, the glycans I think we've covered in the last speech. Only very briefly, I think today, it is

state-of-the-art that you do characterize glycans from specific glycosylation sites and see what is at every single site, and that you do sequence those glycans. That's state-of-the art.

Immunogenicity. I'm not going to spend too much time on immunogenicity, per se, just on the physicochemical parameters that matter in the context of immunogenicity.

Here you clearly have to look at aggregation, look at product related impurities, at potentially immunogenic glycans, at hostile proteins and other process-related impurities, excipients and primary packaging materials that could complex with the molecule, and, of course, also, at leachates, which we have learned recently in a very prominent example.

Then, of course, you can do preclinical assessment. We will have a separate session for this. But you will end up having to do clinical studies for final confirmation.

And, of course, in many cases, post-approval pharmaco vigilant and risk management

programs will be required.

Just a minute on aggregates, because they are really important to look at in detail. You will have covalent and non-covalent or you can have, you will hopefully not have covalent and non-covalent aggregates, soluble and insoluble.

Fortunately, these days, you have a number of orthogonal methods available to look at those. Back in the old days, you usually were using size exclusion chromatography and maybe SDS-page. Now, you have, also, capillary gel electrophoresis and probably most importantly, some of the other modern methods, such as field flow fractionation, and static and dynamic light scattering, not to forget, of course, for the insoluble aggregates, turbidimetry and flowmetry, UV spectroscopy and microscopy.

If you look at aggregation from all of these different angles, the risk that you will actually miss something is significantly reduced to what we were used to in the past.

Biological assays, only very briefly. Of

course, if you do biological assays, they should mimic the mode of action of this product in humans. You can do both in vitro and in vivo bioassays, and you should do multiple bioassays for products which have different modes of action and different indications.

These bioassays should then, of course, also be reflective of these different modes of actions.

This will allow you to establish a link between the physicochemical properties and the PK/PD profile, especially if you do an in vitro assay, where you see all the receptor binding and activation, and an in vivo assay, where you also have the pharmacokinetic and pharmacodynamic parameters.

This is, of course, bioactivity, this is, of course, the first key parameter to predict efficacy.

Now, very briefly, which bioassay should you use? It is, again, a case-by-case and product-by-product decision. You have your ligand

receptor assays on the basic level. Then you have your modern cell-based bioassays with early readout, a lot of discussion on those recently.

You have your classical cell-based bioassays with late readout, and then you have your in vivo bioassays.

Now, the in vivo bioassay is not always the first choice, because clearly they are very variable and you get a limited set of data out of them. They are also very slow, so it's difficult to perform them.

It will be different for a simple nonglycosylated protein compared to a complex glycosylated protein. For a simple nonglycosylated protein, a modern cell-based bioassay might give you much better information than an in vivo bioassay with high variability, because there is no glycans on them that could influence pharmacokinetics and pharmacodynamics.

However, if you take a complex glycoprotein, then you might have to do both a cell-based assay and an in vivo bioassay, to get

both that receptor activation component, also, the pharmacokinetic and pharmacodynamic component.

So there is no single answer. It is, again, product-by-product based on science.

Now, in conclusion, clearly, much more is known today about each particular protein product than at the time of its original development.

Why is that so? Of course, there was extensive knowledge gained by the original sponsor on the processes, on the product, and some of this is available to the general public, but clearly not all.

There is also information in the scientific literature and other domain sources, public domain sources one can look at, and there is extensive clinical experience.

There's specific risk assessments, just thinking about the PRCA assessment we have seen recently, and, of course, there is also data from the development of subsequent expanded indications with these products.

Second, the combination of multiple

orthogonal methods can help overcome the limitations of single methods to obtain the complete picture, I have said that before, and improved analytical methods and design will allow expedited, yet complete, sounds like a paradox, but it's not, development programs, covering all relevant aspects of these products.

And in any case, rigorous standards of ensuring product safety and efficacy must be obtained, while, at the same time, using the best science available at the time, allowing industry to avoid unnecessary and unethical duplication of trials.

Thank you.

[Applause.]

DR. FRASER: I just briefly want to thank our three speakers this morning. We are back on time.

I invite you all to attend this afternoon's workshops. There are two workshops that are going to continue this dialogue and discussion of essentially who, what and how, the

physicochemical characterization and the biological characterization.

As I read the agenda, we are now ready for this morning's refreshment break. So we will be back in this room, I believe, in one-half hour, 11:00.

Thank you very much.

[Recess.]

MR. WEBBER: Just before we start the next plenary session, I just have a couple of announcements.

One is regarding the overheads or the presentation slides. For all the presentations, they will be available on the DIA website at some point after this meeting. There will be a link to that site, as well, through the FDA websites.

I know that I had heard that for Joerg's presentation, there were 300 copies that he brought, but he's having another 200 brought in. It should be available this afternoon, for those who like to have a copy of those earlier.

With that, I think I will turn the podium

over to Dena Hixon, who is the chair for this next session on clinical pharmacology.

DR. HIXON: Thanks, Keith. We have already heard a lot of very good information about characterization of follow-on biological or protein products, and now we are moving on to the use of pharmacokinetics and pharmacodynamics in evaluating a product that purports to be the same as a product already on the market that no longer has exclusivity or patent protection.

We want to be focusing here on the scientific data that would be needed to support that type of application, and we have three speakers lined up, with a very nice range of topics to complement each other.

Our first speaker is Dr. Hae-Young Ahn, who is a team leader in the Clinical Pharmacology and Biopharmaceutics Office in CDER. She has been in that position since 1995, and she is one of the most knowledgeable individuals in CEDR with regard to pharmacokinetics and pharmacodynamics of protein products.

Dr. Ahn's presentation this morning is "An Assessment of Comparability Using PK/PD," and she will provide an overview of the CEDR experience

with evaluating comparability and pharmaceutical equivalence of protein products.

Dr. Ahn?

DR. AHN: Good morning. This morning, I will discuss the assessment of comparability of protein products using PK/PD.

Before discussing the comparability of protein products, I think I made to make a disclaimer statement. The views expressed in my presentation are solely mine and do not necessarily represent the agencies.

My talk will be divided into two parts. The first part, I will discuss the comparability when changes are made in the manufacturing process within the same manufacturer's product. The second part, I will discuss the pharmaceutical equivalence between products manufactured by different manufacturers.

Since the agency has not had much

experience in pharmaceutical equivalence between products manufactured by different companies, I will use the examples of comparability of protein products when changes are made, and those examples, I will make the case of pharmaceutical equivalence.

Protein, peptide, drug, and biologics cover a very wide range, including synthetic peptides, naturally derived peptides, natural, non-blood derived proteins, natural blood-derived proteins, and recombinant products.

Because of limited time, I will limit my presentation to recombinant proteins only.

In general, protein drug products can be divided into two groups, glycosylated protein products and nonglycosylated protein products.

Follitropin and menotropins belong to glycosylated protein products, and insulin and human growth hormone products belong to nonglycosylated protein products.

Since insulin and human growth hormones are relatively less complex, I will focus these two to make the case of comparability.

Insulin, as you know, insulin consists of two chains, alpha chain and beta chain. Alpha chain is made of 21 amino acids, and beta chain 30

amino acids. Insulin exists as monomer, dimer, and hexamer solutions.

Growth hormone consists of 191 amino acids, and it has a single chain. Growth hormone has a secondary structure, as well as total structure.

Current FDA policy for approval of recombinant-derived proteins. In 1986, Federal Register Notice and Points to Consider documents are published. These two documents state there because of unique characteristics of proteins, protein drug substances produced by recombinant technology cannot be assumed to be the same. Therefore, IND must be submitted before clinical investigation on human beings and full NDA, 505(b)(1) should be submitted.

How has the agency determined the degree of similarity of proteins? In the past, because of limited ability to characterize the identity,

structure, and activities of active components, the manufacturing process often defined proteins. Therefore, any changes in the manufacturing process could lead to additional clinical efficacy and safety trials.

However, the development of sensitive and valid assays to characterize the structure and activity has made it possible to define some proteins by physicochemical properties, instead of the manufacturing process.

In addition, the agency has accumulated experience in making determinations of the similarity and sameness of peptides and proteins, when manufacturing processes are changed.

Based on the agency's experience and scientific advances, to characterize the proteins, FDA published a recommendation on comparability in 1996.

In comparability guidance, the following comparability testing programs can be included as a combination of analytical testing, bioassays, preclinical animal studies, and clinical studies,

with the usual provision of complexity from analytical to animal, to clinical pharmacology to efficacy and safety structures.

These comparability tests are not simply a hierarchical system and these are complementary to each other.

Let's move to comparability. When a major manufacturing process is changed within the same manufacturer's product, how comparability can be tested for pre-change and post-change products.

The first example I will use is insulin products. The manufacturing process was changed from process one to process two. The PK/PD study was conducted, with open label, randomized two-way crossover design. Twenty-one health male volunteers will participate in the study, and the reference product was the pre-change product, the test product is the post-change product.

This is the mean insulin and glucose concentrations. Left to right axis represents insulin concentrations, and the right Y axis represents glucose concentrations.

When insulin was injected, insulin concentration goes up, and TMX occurred about less than two hours and then the insulin level starts to

go down.

When insulin kicks in in the body, the glucose level starts to go down and, in about two hours, reaches the minimum, and then slowly returns to baseline.

This is the PK and PD summary table. Reference product was the pre-change product and the test product was the post-change product. Ninety percent confidence interval, ratio of reference to test. AUC and Cmax were very tight, 90 percent confidence interval, what we deemed biochemical criteria that is 80 to 125 percent.

Let's look at the glucose profile. Ninety percent confidence interval for area over the concentration of the glucose and Cmax were tight, as well, between 80 and 125 percent.

The second example is also from the insulin product. A change was made from process one to process two. The PK/PD study was conducted

with a double-blind, randomized, two-way crossover study. Twenty-five healthy male and female volunteers will participate in the study.

This represents the mean insulin/glucose concentrations.

Let's look at the summary of PK and PD. Ninety percent confidence interval for AUC and Cmax were tight, within 80 to 125 percent. However, look at the PD summary table. Ninety percent confidence interval for glucose were outside of the IO equivalence criteria.

By the way, I realize, when we transfer PowerPoint, the scissor sign is supposed to be a plus/minus sign.

Let's move to pharmaceutical equivalence. CFR defines the pharmaceutical equivalence between products. In order to be a pharmaceutical equivalent, the product should have identical active drug ingredients; should have the identical amount of active ingredients; identical dosage forms; and, have identical compendial and other applicable standard of identity, strength, quality

and purity.

Concern for the protein products is identical active drug ingredients. For protein products, to have identical active ingredients is almost impossible. Therefore, our question is how confident are we to determine that two products, two protein products have the same active ingredients.

CFR also defines therapeutic equivalent. If two products are pharmaceutically equivalent and bioequivalent, we determine the two products are therapeutic equivalent and interchangeable. Bioequivalence is sometimes easy, because many protein products are in solution. As long as excipients remain qualitatively and quantitatively the same, we do not have to struggle with the issue of bioequivalence, because these products are self-evident.

So the real issue remains of the pharmaceutical equivalence.

Since the agency was not sure of the pharmaceutical equivalent between two protein

products, historically, until now, the agency has not accepted ANDA submissions and 505(b)(1) ANDA submissions.

How about the future? Can the agency allow biotech companies to submit the 505(b)(2)? With the pharmaceutical equivalence established with the following tests, I will discuss later.

I can say FDA practice has evolved and continues to evolve. A significant amount of experience and knowledge accumulated in recombinant derived protein products.

Therefore, a draft guidance for industry and applications covered by Section 505(b)(2) were published. The draft guidance stated there 595(b)(2) NDA for recombinant derived protein products, and they got rid of that guidance, and 505(b)(2) NDA for Glucagon was approved in 1999.

Therefore, can we accept 505(b)(2)to include interchangeable protein products? In my personal opinion, the answer can be maybe and case by case. If products have the following characteristics, I can say we can have a 505(b)(2),

even interchangeable protein products, if proteins are highly purified, primary structure proven, physicochemical test available for secondary and tertiary structure determination, and if we have clinically relevant bioassays, mechanism of drug interaction is known, and we have validated biomarkers available, and especially if the agency has extensive experience and human data available from multiple manufacturers, I can propose can have a 505(b)(2) or interchangeable protein products.

One example I can say is insulin and growth hormone. Insulin has been on the market and around since 1920, and growth hormone has been marketed since 1950.

This is my proposal for interchangeable or 505(b)(2) protein products. CMC should be studied in order to demonstrate the authenticity of the active ingredient. PK/PD studies should be conducted, because viability may be process-dependent and drug concentration may not be correlated with the biological activity.

Immunogenicity studies should be

conducted, because immunogenicity of molecular variants could be product and process related.

If the sponsor can provide the satisfactory purity profiles, I can say the pharm/tox study may be waived.

I would like to make the following conclusions. When a change is made in the manufacturing process of a given same company drug, analytical testing and bioassays should be conducted and if this analytical testing and bioassay shows some kind of signal to have moderate or substantial clinical impact, animal studies and clinical studies may be necessary, including human PK/PD studies or safety and efficacy trials.

Products may be claimed to be similar to another one already marketed based on the ability of analytical techniques, current manufacturing practice and controls, and clinical and regulatory experiences.

I would like to propose, in order for the sponsor to claim to have a 505(b)(2) or interchangeable product, they should have

analytical testing and bioassays, human PK/PD studies, immunogenicity study, and maybe a pharm/tox study could be waived, depending on the impurity profiles.

These are the references I used for my presentation.

Thank you.

[Applause.]

DR. HIXON: Thank you, Dr. Ahn. Our next speaker is Dr. Raja Velagapudi. He is currently Director of Scientific Affairs at Barr Laboratory, and, in that position, he provides pharmacokinetic support for generic and new drug applications, as well as for development of generic protein products.

For the last 12 years, Dr. Velagapudi has worked in the pharmaceutical industry, providing pharmacokinetic support for regulatory submissions of therapeutic proteins, as well as traditional chemical entities.

Prior to that, he worked at the FDA in the Division of Biopharmaceutics for nearly ten years.

Dr. Velagapudi's presentation this morning, "Considerations in Establishing PK/PD Comparability for Protein Pharmaceutical Products,"

provides a broad perspective on the use of pharmacokinetics and pharmacodynamics in the evaluation of a wide range of peptide and protein products.

He will discuss the usefulness and limitations of PK and PD studies in evaluating comparability and/or bioequivalence of these products.

Dr. Valegapudi?

DR. VALEGAPUDI: Good morning. Thank you, Dena.

We heard from the previous session about the capabilities of analytical and biological characterization in establishing comparability.

In this talk, what I will do is I will discuss the thought process in establishing pharmacokinetic and pharmacodynamic comparability, when we need it.

For those of you that are not in the field

of pharmacokinetics, I will briefly explain that pharmacokinetic studies typically measure the concentration of the drug in the blood stream over the course of time.

Among other things, these measurements tell us about the rate and extent by which the drug is absorbed and, also, how it is eliminated from the body.

Rather than measuring the concentrations, the pharmacodynamic studies instead measure the response of the body to the drug as a function of time.

So the response that is measured could be a change in blood composition, for instance, blood sugar, hemoglobin, white blood cell count, whatever, or a change in other body measurements, such as blood pressure or pulse rate. So those are the things we are talking about with pharmacodynamics.

As you heard from the previous speakers, analytical and biochemical characterization, in some cases, animal studies are needed, are

conducted to demonstrate product comparability.

Sometimes, however, uncertainties about comparability may still remain after these characterization studies.

Human PK/PD studies come into play and become useful tools for reducing those uncertainties regarding comparability that may remain after characterization studies and animal studies.

So the focus is about the characterization studies, that is, regarding the comparability.

Throughout my talk, unless otherwise I specify, when I speak about PK/PD studies, I am referring to the human PK/PD studies, not animal studies.

There are a variety of in vitro and in vivo tools that can be utilized to establish comparability. At the top of this slide, we have physicochemical characterization, which is the mainstay of all comparability testing. This is the most sensitive comparative tool available for the detection of differences between products, as

indicated by the arrow on the left-hand side.

As we progress down the list of comparative tools shown on this slide, the sensitivity of each successful tool towards the product differences diminishes until we reach the clinical studies at the bottom, which are generally the least sensitive method of detecting product differences.

This, however, does not mean that the physicochemical characterization is the only comparative tool of value because of its high sensitivity.

The other question to ask is, for these tools, if a particular comparative tool shows a difference between two products, how likely is that difference to be clinically relevant?

Obviously, if a clinical study shows a statistically significant difference between two products, these differences are likely to be clinically relevant.

On the other hand, because of the high sensitivity of physicochemical characterization

toward minute product differences, the likelihood that minor analytical differences have any clinical relevance is often low, looking at physicochemical characterization.

So this concept is depicted by the arrow on the right-hand side, which shows, as we progress from the physicochemical characterization toward the clinical studies, the probable clinical relevance of any differences found increases.

Characterization invariably starts with physicochemical characterization, which is the most sensitive one. If no uncertainties are found at this level, no further studies are warranted.

If, however, sometimes uncertainties about comparability remain after this step, we proceed down the ladder to the next comparative tool that would be expected to help resolve the uncertainties we found.

The human PK/PD studies are intermediate, both in sensitivity toward the product differences and, also, in the clinical relevance of those differences found.

In this respect, they become very useful tools to help resolve any remaining uncertainties about comparability following our characterization

studies.

The complexity of the protein comparisons can be viewed as a continuous spectrum. Throughout this morning, all the speakers have explained to us that there is no black-and-white magic box in protein comparisons. It is a continuous spectrum.

I want to point out that the collection of comparative tools needed for a particular situation depends on the complexity of the comparison, not necessarily the complexity of the protein.

In general, the simpler the protein and the closer the match, the simpler the comparison.

When more complex proteins are compared and you have greater differences between the products, then the complexity of the comparison increases.

The character of tools required to establish comparability may change from characterization alone for simple proteins, with

the simple comparisons, on the left-hand side, and may go up to using all available tools, including clinical studies, in the most complex comparisons, as shown on the right-hand side.

However, I believe that the majority of the cases will fall somewhere in between these two extremes and will often require characterization plus some human PK studies to collectively reduce the uncertainty that was found in comparability studies to an acceptable level. As I see characterization, where necessary, human PK studies are the comparative tool that will be used most often.

I would expect, also, those types of studies shown on parentheses, such as animal studies and PD studies, would be used less frequently and only in those cases where they are necessary and appropriate.

One of the stated questions that I was supposed to ask is can the bioequivalence of a protein solution be considered self-evident.

My belief is yes. The bioequivalence may

be self-evident, if all the criteria are met; that is, the product is a solution at the time of administration; the product quality attributes are comparable. That means, in characterization, you have no quarrel about identity, comparability. And the formulations are qualitative and quantitatively comparable, and the dosage delivery devices are comparable, and mode of administration is the same.

When all these things align, yes, I think it will be self-evident. Knowing these issues and comparing the protein products from different manufacturers, I would expect to see this happen very, very infrequently.

However, because of the need to avoid unnecessary human experimentation, it is very important to remain cognizant of the fact that for some solution products, bioequivalence may be self-evident, making it unnecessary to conduct human PK studies.

The concept is still the same. Do not do it unless it is necessary. Do not expose the humans unless needed.

For solutions for drugs, the provision to meet bio requirements to conduct comparative in vivo bioequivalence studies is codified in 21 CFR

320.22. FDA routinely grants such waivers for small molecule products, meeting the criteria on this slide.

This is done for the process changes, as well as abbreviated new drug applications for generic products.

So this is not a new concept; however, we have to explore that for proteins.

When do we need a PK study? As I stated earlier, I believe that in many cases, we do need to do a PK study. PK studies are necessary if uncertainty regarding comparability remains after characterization and there is no suitable animal model that predicts the human responses and differences.

Also, if uncertainty remains after animal PK study, that means animal PK study is not required at this point, but if somebody has done it and they found some remaining uncertainties, you

can go on to the human PK to resolve those things, or if the product is not a solution, I just put, as an example here, suspension, most of the proteins are either solution or suspension, so it could be any other product other than a solution that needs a PK study.

Is a PK study always feasible? Human PK studies are generally feasible for most systemically available proteins. So if it is absorbed, if it is in the blood, yes, the methodology is available, PK can be done.

A variety of highly sensitive and selective bioanalytical tools, ranging from advanced instrumental technology and to the bioassays, immunoassays, are already available for the development of validated bioanalytical methods.

In some cases, cell-based assays were used, but not frequently. Variability in PK parameters of protein products, while they are on the higher side, is still generally manageable.

When should we not do the PK studies? On the other hand, human pharmacokinetic studies

should not be conducted in those cases where little or no uncertainty in comparability remains after characterization under animal studies.

The PK is not something that we do it for fun. There is a reason to do that. That reason was the uncertainty that was raised during the characterization studies.

Also, the second case is the PK studies are not simply feasible. Why? Because there are some locally acting drugs that you will see no measurable levels already fully absorbed that we may not have to do PK studies, or the assay technology probably is not available for that molecule to actively pursue.

The other one is there is a safety issue, ethical issues, exposing to the human. So in those cases, we may not do the PK studies, because they are not feasible.

The third one is if relevant animal models exist to distinguish the differences in humans, then we may go with the animal model when a relevant model is available, then we are not forced

to do the PK studies in humans.

Finally, what we d is we should use it, whenever it is possible, instead of conducting the human studies, whenever we have the animal models.

When should animal PK/PD studies be conducted? This is the other side of it. If somebody comes up and says, "When should I do an animal study," we should look into the possibility of doing animal PK studies when uncertainties in comparability remain after characterization, that is always there, and where there is an animal model that exists which is predictive of the differences in humans.

That means not only that it is a correlation, but there is also some kind of a sensitivity that it can be correlated to the human sensitivity.

In addition, animal PK studies may be appropriate for resolving uncertainties in comparability where PK testing is not feasible or not advisable due to high risk.

And there could be ethical issues, not

only safety issues in humans. Animal PK/PD studies may not be needed often. There are cases where they are useful tools to minimize uncertainties in comparability between manufacturer comparisons.

So even though the animal study looks like we may not be doing it that often, still there are cases they find very useful for between product comparisons.

So the limitations in interpreting the PK studies. Even though pharmacokinetic studies are useful and commonly used comparative tools, they are not without limitation.

Like any other technique, any other tool, we will have some limitations.

These limitations are equally applying to brand and generic manufacturers. So when you're talking about limitations, it's the limitations of the PK, not limitations of a certain manufacturer.

For example, a statistically significant difference in pharmacokinetic measurement due to relatively high precision does not necessarily mean that the difference is clinically relevant.

So you may be doing PK studies and found differences, statistical rigor, but may not have clinical relevance.

In some limited cases, pharmacokinetic responses may be somewhat less sensitive than the pharmacodynamic response, possible.

Despite the shortcomings of these special cases, rarely you will see these things. However, pharmacokinetics remains one of the most valuable tools available for between manufacturer comparisons.

Next, I want to briefly touch on the pharmacokinetic design considerations.

The pharmacokinetic characters of the brand product generally dictate the design of the human pharmacokinetic studies. Nobody is going to go and design a study blindly. You look at the label, you look at the literature and the background, whatever it takes, and the you know the brand product pharmacokinetic characteristics and then you design PK comparability studies.

For example, risk-benefit on a drug

substance interaction may preclude studies in the normal, healthy volunteers. In a few cases, high variability and low drug levels after a single dose may necessitate multiple dose studies.

In cases of long half-life, you may end up doing parallel studies. The route and method of administration has to be the same and the injection site has to be the same.

One thing you have to realize is if you are injecting abdomen, thigh or forearm, pre-specify before the study where you want to do that. After the study, it will be moot.

Sampling during the study, at least three half-lives is desirable, consistent with the guidance that we have to cover at least 80 percent of the AUC, the area under the curve.

The use of truncated area is also acceptable in the case of long half-life drugs, when you don't have any other alternative.

These design considerations are similar in many cases to those found in FDA guidances for the small molecules. We are not inventing anything

new.

Can the standard 90 percent confidence interval that we use for bioequivalence criteria be used for proteins?

In most cases, the bioequivalence criteria for small molecule drugs can be used for protein drugs, also. For those of you who are not in the pharmacokinetic area, I will explain a little bit about what this confidence interval is about.

This criteria is mainly applied to two parameters, the maximum concentration, Cmax, and, also, the area under the concentration time curve, AUC. Because we are dealing with comparability, we calculate the ratio of the Cmax of the one product to the other, and, likewise, the ratio of the AUC, of one product to the other.

Based on the number of people and the variability of the drug, we can calculate the confidence interval around this ratio and the ratio has to be within 80 to 125 percent.

That doesn't mean that the mean ratio is between 80 to 125 percent. Let's not mistake that.

The variability allowed is not in the mean. It is the confidence interval band that is allowed.

Normally, the vast majority of products that meet the bioequivalence criteria actually show the average ratios within 90 to 111 percent.

So you are talking about ten percent in the mean, but you are talking about the confidence interval being 80 to 125 percent. So it is relatively very conservative criteria to show the comparability.

The bottom line here is that exactly the same bioequivalence criteria that are used for small molecule products can be and are routinely applied to protein products, as well. We want to be sure that the products are comparable.

Then when do you use a pharmacodynamic study? In addition to the pharmacokinetic measurements, in some cases, pharmacodynamic measurements may be provided, and these provide more useful information regarding the clinical comparability.

We are now moving on from the exposure to

the activity or clinical relevance. The PD is more closer to the clinical relevance than PK, because of the bio response involved.

PD measurements may be valuable if they are clinically relevant and they are feasible. Also, PK is less sensitive than PD measurements can be used, which is the way it appears.

Also, if PD measurements would address the uncertainty remaining after PK studies, we could do that.

In any case, if the PD measurements are to be included, one thing to remember is try to design a study with a simultaneous PK/PD end point in the study, which is most preferred.

The reason for that is that if you do a PK alone and you have uncertainty, then you are running into PD to solve it. Sometimes PD has some uncertainties or criteria that you cannot set well, then PK will help.

So this understanding of the relationship, as well as two parameters comprehensibly produce the uncertainty.

This slide shows the likely pathways that one would follow in the event that some sort of PK/PD work is needed to address uncertainties that

may arise from the comparability studies.

What we are showing here is you have a likelihood of either doing a PK study or going towards the PK/PD knowing the characteristics of the drug. Very rarely, you will also see, if PK is not available, people going directly to the PD or, first, they do the PK study and then realize uncertainties were not resolved, then they go to the PD.

At that point, if PK or PD or PK/PD did not resolve, then most likely one will proceed doing some targeted clinical studies. Targeted clinical studies are not black-and-white, go and do the safety/efficacy studies. It is to resolve the uncertainty that arose up to that point.

So they are targeted in the sense to answer the questions, not to blindly do the study for the sake of it.

What additional information will a PD

provide? Pharmacodynamic studies will help minimize the uncertainties remaining after pharmacokinetic studies. Pharmacodynamic studies are reflective of the human response to molecules over a series of time intervals.

Therefore, PD studies that utilize clinically relevant markers may eliminate the need for separate clinical safety/efficacy trials.

So there is some economics involved here and relevance to do the PD studies.

Pharmacodynamic end points are generally chosen based on their relevance to the clinical outcome and measurability. The area under the effect curve, AUEC, and the Emax, which is the maximum effect, and time to the maximum effect are commonly measured.

In special circumstances, other parameters can be added based on the drug application and measurability. So those parameters are inclusive, not necessarily mandatory.

The statistical criteria for bioequivalence may be upright in cases of low

variability and if you hit the high variability with PD parameters, three different alternate criteria may be more appropriate and applicable. It may have to be discussed with the agency.

Next, I will come to the point of interchangeability, which is the hot topic for a lot of speakers. I will come from my view of knowing from both sides what interchangeability means and how we can approach it.

Next, I want to tell you about how I envision this one. I envision that some products filed via an abbreviated pathway may also be eligible for interchangeability. Do not mistake the idea that every application is targeted for interchangeability ahead of time.

You are submitting an application for an abbreviated pathway through the process, interchangeability will emerge based on the merit of the comparability.

As in the case with small molecules of drug products, a determination of interchangeability between products would be based

on the degree of comparability between the products, there is no mistake.

The concept of interchangeability is not at all foreign to the protein products. The last bullet shows a few examples of protein products that were given interchangeable status by the FDA.

Some may argue it was done a long time ago, it was a mistake, but to mine, there were no instances of anything wrong with it. They are working fine and they are on the market.

The next thing I wanted to point out to you, interchangeability of protein products. When considering the types of studies needed for the support of interchangeability, the first thing that you have to do is see what is the goal.

The goal is to minimize uncertainty about therapeutic comparability. I believe that in most cases, characterization and PK/PD will provide sufficient evidence to support interchangeability.

So that means that any studies leading from analytical comparability up to the point of PK/PD comparability probably will be in that

package to support interchangeability.

Less frequently, targeted clinical studies might be needed. The reason for that is when we are doing the submission of the package, there will be some uncertainties that are not resolved up to the point of PK/PD comparability.

Those issues may be specifically addressed in the clinical trials. Again, I say doing clinical trials is not for fun. It has to be addressing the unresolved issues that were raised from the characterization studies or PK/PD comparability studies.

So in summary, PK studies are feasible for the majority of the proteins. PK studies provide information about comparability and systemic exposure. PK/PD studies may not be needed for solution or protein products that are comparable analytically. This, I mean, is you are comparing to the point of no doubt.

PK studies are generally necessary if uncertainty about comparability could not be adequately minimized through the characterization

and animal models that are relevant.

The standard 90 percent confidence interval BE criteria are appropriate for most PK studies. If PD measurements are to be included, simultaneous PK/PD studies are often preferred.

PK/PD studies in conjunction with the adequate characterization are usually sufficient to support approvability of the abbreviated package, and, in some cases, interchangeability.

With that, I thank the organizers of this DIA and FDA for giving me this opportunity, especially Dena Hixon, who coordinated and cooperated with us and is an excellent guidance. Also, my co-speakers, Hae-Young Ahn and Mark Rogge.

Thank you.

[Applause.]

DR. HIXON: Thank you, Dr. Velagapudi.

Our final speaker for this session is Dr. Mark Rogge. Dr. Rogge is currently Vice President of Development at ZymoGenetics, where he oversees pharmacology and safety activities for research, preclinical and clinical development.

He previously served as Vice President of Pharmacometrics and Preclinical Development at Immunex.

Dr. Rogge has authored numerous publications on bioequivalence, drug interactions, basic preclinical and clinical pharmacokinetics, pharmacodynamics, and formulation factors that influence drug absorption.

He is also co-editor of the soon to be released book "Preclinical Drug Development."

Dr. Rogge's presentation this morning, "Complex Protein Therapeutics, Pharmacokinetic and Pharmacodynamic Considerations," will further discuss the use of pharmacokinetics and pharmacodynamics in evaluation of complex protein products and provide a closer look at sources of variability in PK and PD profiles of complex proteins.

Dr. Rogge?

DR. ROGGE: Thanks, Dena. Thank you very much. It's great that so many people have come to attend this open forum. This is such an important

topic, and, looking at the name tags, I can see that people are really coming from a broad swath of the industry here, and this, I think, in the end, is going to make the quality of the products at the end of the day much, much better.

What I would like to talk about I think will be very complimentary to the previous couple of presentations that were given. I would like to provide a perspective on pharmacokinetics and pharmacodynamics from an innovator's environment. How do we use these evaluations throughout the early and mid and late stages of clinical development, as well as once the product gets out into the marketplace?

There are a lot of comparability evaluations that we conduct along the way, and PK and PD can provide a very important component to better understanding that comparability.

Again, this is from an innovator's perspective, the world that I live in. Generally, PK and, occasionally, pharmacodynamics are considered a requirement when we conduct

comparability evaluations, when we go to a new process, when the product is in clinical development, and almost essentially always when the product is out in the marketplace.

Oftentimes, we're not required to do any kind of PK evaluations if the product is in a preclinical stage of development or it's a non-IDE-enabling trial.

However, we will usually do the studies anyway, because we are trying to get a handle on what the sources of variability are, as the components of this ensemble of the product, as it was described earlier, may change during the process change.

So how do we determine the rigor that goes into the amount of PK and PD work that we are going to be conducting in any of our comparability evaluations? For the most part, it is predicated on patient risk. The more risk, as you can imagine, the greater the need for there to be some form of comparability evaluation along the way to ensure continuity between the current product and

that which we intend to move towards.

How are we going to measure risk? Well, it is primarily patient risk, the severity of the disease, the number of doses the patient may get during that comparability evaluation, the duration of dosing, and something that is not always, I think, considered is patient oversight.

In a phase one or a phase two trial, there tends to be a fair amount of patient oversight that is occurring throughout the dosing period, the study period. Out in phase three and most definitely in a post-approval period, there is much less patient oversight and, hence, the likelihood at least of greater risk.

There are other elements of risk that need to be considered here, as well. The molecular complexity, and we have had nearly a whole morning talking about the complexity and I will be getting into that a little bit later, so I won't dwell on it here.

The product heterogeneity. I like that term of these products being ensembles of major

components and minor components and then other components that we don't know if they are major or minor, at least in terms of their qualitative aspect of what they do, and we will be bringing up some examples here in a few minutes to talk about that.

What is the robustness around the composition of these products? If something changes in terms of its quantity, one of these isoforms within the process, is that going to have any impact on the kinetics of the drug, the disposition of the drug, where it goes in the body?

And, of course, the stage of development and the CMNC product precedence that is associated with it. How much experience do the manufacturing folks have with making this product, with using this type of process, or at least working with similar molecules?

Well, there's a multitude of ways in which we can approach PK and PD, biodistribution types of evaluation to assess comparability. It tends to be very science-driven, as you would expect. There's

a lot of historical precedence, also, that is built into the system.

But in the end, it is generally very much tailored to the situation at hand. We can either do non-human studies or human studies. The non-human PK studies, just simply looking at blood concentrations, for example, can provide a fair amount of insight. It can take some of the risk out of, at the very least, the decision-making that we're going through as we're considering the transition to a new product.

Biodistribution studies. I'll be putting up some examples here in a few minutes that illustrate biodistribution data. Biodistribution studies can provide an incredible amount of insight into what is going on with the product or maybe even some component in that ensemble.

There are human PK and PD evaluations, of course, that we can do, and the previous speaker talked about them. Yes, they are expensive. They do provide a lot of insight. They can provide a lot of value, and they are warranted in many cases

at least in the innovator's world.

There are factors, though, and some of them were talked about. Disease state can have a profound impact on how these molecules behave when they get into the body.

What type of study are we going to do? Is it going to be a crossover trial with that standard 80 to 125 bioequivalence criteria applied or do we do some type of parallel group trial and maybe do repeated measures, trying to understand the variability of the performance of our reference molecule, and see if the variability of the test molecule we're moving towards falls within that variability.

I don't know if the variability is 80 to 125 or it's 90 to 110. It may not matter. It may be okay for it to be 25 to 175. We can gain a lot of insight by doing this the proper way.

From a statistical standpoint, there are opportunities to move away from the standard parametric analyses, if we would like, and go into non-parametric analyses.

The previous speaker talked a little bit about the normal distribution of the data or at least the ability to log transform the data, do

some kind of transformation to create a normal distribution of your end points, AUC, Cmax, whatever.

If you are able to do that, that's great. That does allow you to do more of a traditional or parametric type of evaluation. However, if you can't, take the liberty, take the opportunity to do something at least from a statistical standpoint that makes a little bit more sense.

Do some form of a non-parametric analysis, and those have been done successfully, working together with the agency.

So in this context of how we are moving toward characterization of new molecules as they are moving through clinical development, as these products are evolving in the form of process changes, scale changes, whatever, we are trying to understand some of the sources of variability, where do they come from, what is acceptable to

allow to drift or move, what is not.

I would like to begin moving toward some examples to help you understand where those sources of variability might be and some of the types of studies that can be done to help understand that.

Two types of variability here are biovariability, those of us in the PK world occasionally call it, exists, being intrinsic and extrinsic. Now, for the most part, this morning we have been talking about intrinsic sources of biovariability, the size and the shape of molecules, the charge that's on them, the carbohydrate structures.

We haven't beaten up carbohydrates enough yet this morning. I'm going to talk a little bit more about them, but I won't dwell on them, I promise.

But there are some very significant qualitative and quantitative issues to consider when we do have process changes in terms of how they may affect the performance of the drug in the end.

Over on the right-hand side, you can see we have extrinsic sources of variability, such as the route of administration, the formulation. I

will giving you an example at the very end of my presentation on formulation aspects and where you might not think it could affect the kinetics or dynamics of the molecule, in fact, you will see from some published data that, indeed, that was not the case.

Dr. Kozlowski gave a very complicated example of how severe the number of permutations can be with a glycosylated monoclonal antibody. I either didn't have enough time or access to the best statisticians out there or something to do something similar.

My example is a little bit more simple than that. I created an imaginary molecule protein that simply had three glycosylation sites. I won't dwell on this very much, other than to say that if it is fully glycosylated, there's upwards of 84 permutations or glycoforms that could exist.

If we allow some of those glycosylation

sites to go empty, to not be occupied by one of the three carbohydrates that I laid out here, we can get nearly 300 glycoforms within this potential ensemble, some of them major, no doubt, some of them minor.

Again, I would like to impress that just because it may be minor, it is not necessarily going to have an insignificant impact on the performance of the drug.

Now, this is an example of some work published a couple of years ago on calcitonin. The authors had glycosylated calcitonin, a variety of different ways. I believe there's 17 different glycoforms here.

I'm going to point on the middle screen, and I hope most of you at least can see it, but over on the right-hand side, this right-hand column, we have receptor binding activity and there's anywhere from, I believe, about a five-fold to a 250-fold difference in the receptor binding activity across these glycoforms and roughly, I think, about a two to seven-fold amount of

variability in the hypocalcemic activity.

When these different molecules were given to animals, how much of a reduction in serum calcium occurred?

If you would expect that higher receptor binding activity was going to result in a greater ability to reduce serum calcium, you're wrong. It actually doesn't.

If you look at a correlation, the R-squared is 0.1754; in other words, about 17.5 percent of the variability in the receptor affinity could be attributed then later on to the hypocalcemic activity.

Where is that other 82, 83 percent of the variability coming from? It is probably, at least in part, due to pharmacokinetic reasons.

In fact, the authors did do some work to try to understand whether or not disposition of the molecule was in any way affecting the hypocalcemic activity, and, indeed, that was the case.

If you look at the lower curves on this figure, now, this is uptake into the liver, the

lower curves, those animals tended to actually have the highest amount of hypocalcemic activity.

Well, one might think, intuitively, it makes sense, because there's now more of the product circulating and, therefore, will be available to exert its pharmacological effect, and vice versa; those with the highest concentrations in the liver tended to have the lowest amount of hypocalcemic activity.

We can explore this a little bit further. Some very nice work was done across a series of papers by Elmagbari and his colleagues, as published over the last several years, and they took encalphalon analog and glycosylated it nine different manners, and they looked actually at the differential binding to two opioid receptors to see if there would be a consistency. If you increased binding to one receptor, would you, in turn, increase binding to the other?

Why is this important? Because most of the products at least I have worked on do have more than one binding site. Products I'm working on

right now have more than one binding site; not only pharmacological binding sites, but to carrier proteins, to binding proteins that are there naturally to neutralize these glycoproteins that we're administering.

So in this example, was there any correlation between the changes in binding to the delta receptor with binding to the MU receptor? Some correlation. I wouldn't call it a good correlation. About 63 percent of the variability in the delta binding affinity was correlating with the MU affinity, but, again, there was something else going on here.

The glycosylation was impacting how these molecules were preferentially binding to each other. There was some level of independence there.

Probably what speaks most at the very end of the day is did they behave the same, did they work the same; who cares if there was some difference in the binding between these two receptors.

Well, there was difference, actually, and

the figure on the left is a monosaccharide version of the encalphalon. The figure on the right is a disaccharide version.

Let's go back to the left curve. You can see that there's about an 80 to 85 percent degree of analgesia that was produced in these rats, I believe this was done in. The dose was 20 milligrams per kilogram and the pharmacological effect persisted out to approximately 90 minutes post-dose.

If we go over to the right-hand side, the disaccharide variant, we are getting upwards of 95 to a 100 percent analgesia and the analgesia is persisting out to about a 120 minutes post-dose.

On top of that, the disaccharide was only given at ten milligrams per kilogram, half the dose, yet is producing twice the pharmacological effect and even more than that, the amount of analgesia is even greater than that with the monosaccharide.

What is some explanation underneath that? I wasn't able to go into the papers and find these

type of data for those two that I just gave you, but they did have some data published for two other variants that were in their publications.

Now, one was the encalphalon, the opioid peptide alone, and that's in the top row here. Then there was an old glycosylated peptide and that was just a beta glucose variant. It was a monosaccharide.

What they found was that the blood brain barrier permeability was two-fold greater with that monosaccharide than it was with non-glycosylated variant alone. In other words, twice as much product was able to get into the brain at the site of action when it was glycosylated as when it was not glycosylated.

In addition, if you look at the half-life, glycosylation stabilizes the molecule. It slowed down its clearance from the body. Half-life was approximately two-fold longer with that glycosylation form.

It would have been interesting if they were able to pull similar data, generate similar

data for some of the other variants, as well, to give a better picture, but I think it's clear that glycosylation can have a significant effect.

Again, when we are talking about ensembles of molecules, five or ten or twenty percent of something, if it gets reduced down to one percent or if something doesn't exist at all and it now becomes five percent, could have a relatively significant impact on the activity of the drug, safety, as well as efficacy.

Now, I've talked a little bit about biodistribution here and I would agree with the previous speaker, Raja, that we need to be judicious, we need to be very smart in how we use animals in the evaluation of these products. It shouldn't be done in any kind of willy-nilly manner.

But at the same time, the data that can be generated from these studies can be invaluable, particularly for those of us in the innovator world, when we're trying to understand how these molecules are distributing, where they're going,

how much stays in any organ, and, for that matter, may even exert a pharmacological effect or not.

This is some work published quite a long time ago, you can see, almost 20 years ago, on GM-CSF. The authors created five variants of GM-CSF, and I'm just going to call them one, two, three, four, five, because I'm not going to be able to point to all the screens in here.

What I would like you to think about is the mass balance element here. If it's not in the bloodstream, then it's somewhere else. If it's in the bloodstream, then it's not somewhere else.

If we look at variants one and two, the top two lines, and go over to the plasma, you can see that at this time point, published in this table, 16 percent of the drug was in variant two, whereas 25 percent was in variant one.

Go over to the kidney, and you can see that the concentrations were roughly the same, but in the liver, they're a little bit higher and, in fact, in the lung, they're a little bit higher. Well, that is consistent with the mass balance.

However, let's go to variants two and three, where, again, the plasma, we see a reduction, but in the kidney, we see a reduction

almost three-fold. It's only about 30 percent of the variant two version. There is about a 50 percent reduction in the liver, and we've only got about 25 percent of the amount in the lung as we have for the variant two.

So, clearly, the drug was now starting to go somewhere else or maybe it was being cleared faster as the variant moved from glycoform two to number three.

I'd like to make one last point off of this slide, also, and it gets back to pharmacokinetic studies and their value and understanding what you know and what you don't know.

If we look at variants one and five, the top and the bottom, plasma concentrations are, for all practical purposes, indistinguishable, 25 and 28.

Yet, you look at kidney, where the

corresponding concentrations are 22 and 12, liver 7.5 and 52, you need to be careful. It could have been potentially over-interpreted or misinterpreted that there was no difference between these variants, when, in fact, there was, at least in organs or certain tissues, significantly different concentrations.

Glycosylation is not the only source where we can get this kind of variability. There are other means in which it can come about, as well.

In this case, some work was published looking at the charge on the molecule, the isolectric point on it. What the authors did is they took avidin, which has a relatively high isolectric point, I think it's around nine, nine and a half, and they neutralized it.

Following the neutralization process, the PI, the isolectric point had come down to about five and a half, I believe. You can see from here that there is anywhere from, roughly, two to 20-fold differences in the change in tissue or organ distribution between these two molecules,

and, again, considering that a minor variant may not necessarily be minor, if, in some way, that variant has a much greater ability to get into a tissue and so something.

I'd like to talk about one extrinsic source of variability here, and Raja, the previous speaker, had brought this up in his presentation, as well, and it has to do with formulation.

Recombinant human interferon beta-1A is marketed in the United States as two different brands, Avonex and Rebif. It's the same product, the same active ingredient. The difference, though, is in the formulation. Avenues contains 15 milligrams per mil of HSA, PH7.2 and phosphate buffer. Rebif, on the other hand, has a little bit less HSA in it, PH is lower at 5.5, with acetate buffer, and it also contains some mannitol, as well.

This isn't at least what I thought initially to be a big difference in formulation, but following a crossover study that we did in healthy volunteers, where we looked at the serum

interferon activity, as well as the pharmacodynamic marker, standard PD marker for interferon, neopterin, indeed, there was a very significant difference, substantial difference in the performance of these two molecules.

The serum interferon activity from the Avenues formulation was over two-fold greater than for the rebif formulation. That translated downstream into a 44 percent increase in the PD activity of the molecule, as well.

So I don't think we should discount formulation. I know some comments were made earlier, as well, about if it's in solution, we may not have to do a PK study. Both of these formulations were in solution.

So just some quick, very quick summary thoughts. Organ and tissue distribution studies can provide a lot of insight. PK studies, nonclinical studies, can provide a lot of insight. Make sure they are in relevant species, though. It is vital that it be in relative species that mimic the human physiology in many ways.

PK studies in humans can provide insight, but you've got to understand their limitations.

PK is most useful when it's correlated

with a clinical outcome. At least early on, I'm not always sure what a five, ten, or maybe even a fifty percent change in some PK parameter means, an AUC, a Cmax or whatever.

It is only when it is correlated with some outcome or at least a risk of a change in safety or efficacy occurring that we can understand what that PK honestly means.

Also, pharmacodynamic parameters, very valuable, absolutely valuable. If you can do them, do them. Do them for safety, though, as well as efficacy, but I would not dissociate PK from PD.

PK should always be conducted with these molecules. If you can do the PD on top of it, so much the better.

Those are my comments. Thank you for your attention.

[Applause.]

DR. HIXON: I want to thank all three of

the speakers very much for their time and effort that they have put into providing these presentations for us.

I do want to welcome everybody to attend the breakout session this afternoon, at which time there will be an opportunity for questions, comments, and further discussion on pharmacokinetics and pharmacodynamics.

Keith?

DR. WEBBER: Well, don't run off quite yet. In organizing the meeting, we wanted to try to get an opportunity to have Dr. Janet Woodcock give us some words, as well.

Fortunately, she is here and just prior to lunch, she would like to come up and talk to you a little bit about future plans.

DR. WOODCOCK: Thank you. Good morning. I know I stand between all of you and lunch, and so I will be brief.

It is great to see a good turnout at this meeting. We have begun to discuss these scientific issues, I think, at a greater level of specificity

and we will continue to do that over the next day and a half, and I think that is very important for the FDA as we move forward.

This is, of course, a scientific meeting and we're not talking about policy or where we're moving, but I would like to give everyone an update, because I know everyone is interested in that.

We hope, from the agency, to be able to issue our background document within the next several months. That is not a policy document. It is going to be a discussion of the prior regulatory and scientific steps that we have taken regarding protein molecules over the past 50 years or so, and there have been many steps that have been taken over those years, and that should provide a good overview for any further steps that we take.

Subsequent to that, we plan to issue, at some point, as we have already said, a draft guidance and we are working on all these documents, and that would be for comment by the entire community.

That guidance will be more informed by the scientific discussions that are going on here. It may not be a single guidance, but a set of

interlocking guidances that touch on the different scientific areas that are being discussed at this meeting and the different scientific controversies that need to be resolved.

So we hope we would have that done within a reasonable amount of time, I can't give a time frame on that, as a draft for comment, and, at that time, we will probably provide another opportunity for public discussion of the proposals within that draft at that time.

The background document probably would simply be something people could submit comments on, but it will be more factual and descriptive of past actions.

Everyone is watching what FDA is doing in this regard very closely and we want to have an open and transparent process. I'm glad there are so many people here, because this is very open and transparent, and we will continue to provide

information to people in what we're doing and the next steps that we are taking.

So thank you very much, and I wish you very good scientific discussions over the next several days.

Thanks.

[Applause.]

DR. WEBBER: Thank you, Janet. I guess at this point, I would like to certainly thank all the speakers from this morning's plenary sessions. I think they have given us a great solid background and baseline from which to initiate our discussions this afternoon.

Again, there will be four breakout sessions running concurrently. Each one will run twice. So if you can't decide which one to go to, you can pick at least two and perhaps move between them.

This room will be broken up into four rooms. There should be labels on each one that tell which session is occurring in that room.

So at that point, there will be boxed

lunches outside. You can grab your lunch and sit and talk with your colleagues, find a place to eat, and then we will meet back here at 1:30.

Thank you all.

[Whereupon, at 12:23 p.m., the session concluded.]

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